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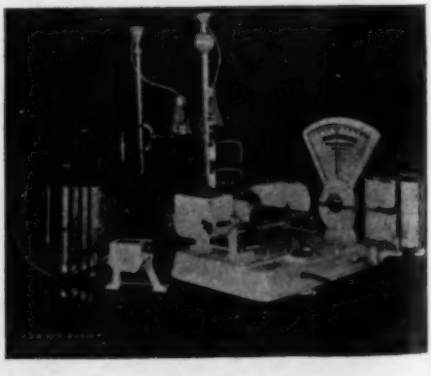

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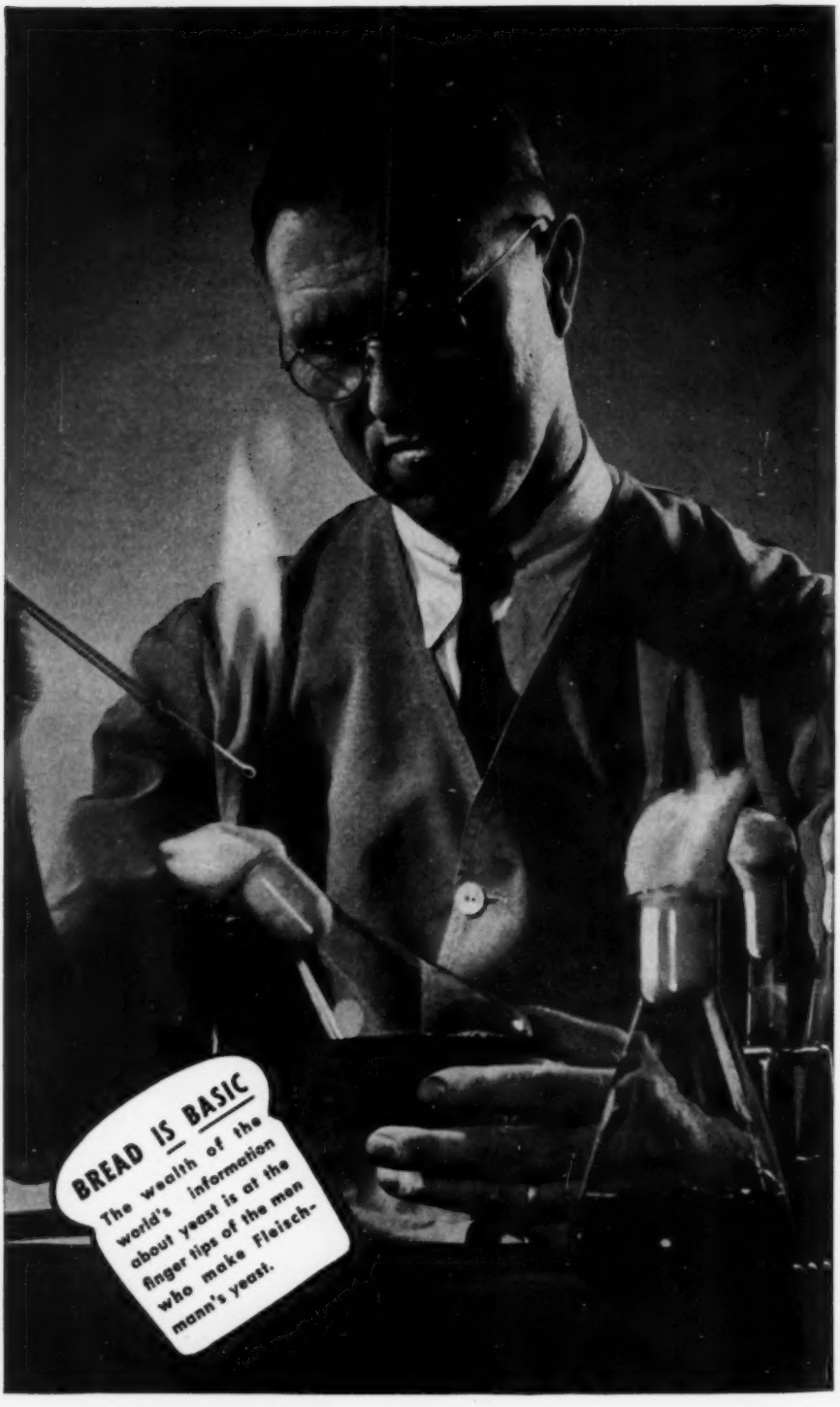
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CEREAL CHEMISTRY

VOL. XX MARCH, 1943 No. 2

THE NUTRITIVE VALUE OF WHEAT GERM PROTEIN

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Pillsbury Research Laboratory, Minneapolis, Minnesota

(Read at the Annual Meeting, May 1942)

Wheat germ has had some use as a human and animal dietary supplement because of its high thiamin and flavin content. However, little attention has been directed toward the use of wheat germ as a protein source although it contains as much as 30% protein. Many attempts have been made, some more or less successfully, to incorporate wheat germ in the baking of bread; but unless the germ is specially treated, more than 2% in the flour has a marked detrimental effect on loaf volume and color. The use of germ as a bread supplement has been motivated by its vitamin content and not primarily by its protein supplementing value.

As far as we are aware no one has investigated the nutritive value of wheat germ protein for the growth of young animals. Boas-Fixsen and Jackson (1932), and Chick, Boas-Fixsen, Hutchinson, and Jackson (1935), using the nitrogen equilibrium method on adult rats, have reported that the proteins of wheat embryo are of slightly superior value to those of the endosperm, and are about equal to whole-wheat and yellow-maize proteins.

In this paper we present evidence that the protein of wheat germ is of as good quality and as good supplementation value as the proteins of skim milk powder and certain other animal proteins. The method used for determining the biological value of proteins is essentially that of Osborne, Mendel, and Terry (1919). This method involves the growth of young rats on a basal ration free from protein, but otherwise nutritionally complete, to which has been added the equivalent of 10% of protein in the form of the food product to be tested. The biological value of the protein is calculated as the ratio of the body weight gain to the amount of protein ingested during the experimental period.

Experimental

The basal ration: The composition of the principal basal ration used in this work is as follows:

Protein source under test	X%
Sucrose	(90—X)%
Salt mixture	4%
Corn oil	4%
Cod-liver oil	1%
Liver extract powder 1–20	1%
Thiamin	3 μg/g ration
Riboflavin	3 " "
Pyridoxine	6 " "
Pantothenic acid	15 " "
Choline	1000 " "

The only protein in this diet, other than the protein under test, is obtained from the 1% liver extract powder 1–20. This dried water extract of liver, containing 8% nitrogen (which is equivalent to 50% protein) will furnish 0.5% of crude protein to the basal ration. Although ideally the basal ration for protein quality work should contain no extraneous protein, there is reason to believe that the error caused by this inclusion is insignificant. In the first place the "protein" of this liver extract powder is very poor in quality, as was shown when a group of four rats were placed on the basal ration in which 20% of this liver extract powder was the only source of protein. After four weeks the average gain in body weight was 5 g, and the average food consumption was 190 g, indicating a biological value for this "protein" of 0.25. The chances are slight that a protein of such poor quality will have any significant supplementing value at 0.5% in the ration.

A second, and more important, reason for believing that 1% liver-extract powder in the ration causes no significant error in the biological values of the proteins under test is apparent from a considerable number of trials in which the liver-extract powder was omitted entirely from the basal ration. Some of these results are presented in this paper (Table I); they represent assays of the test proteins at 5% protein levels.

At the 10% germ-protein level without liver extract a fair percentage of the rats developed a severe hemorrhagic disease characterized by sudden onset, marked weight loss, severe anemia, and in most cases death within four days of onset. This seizure almost always occurred in animals which in the previous week or two had shown the most rapid growth and the greatest food consumption. From rats which failed to develop this condition it was evident that the biological value of the germ protein, as determined on this ration, fell well within the range of variation of the values that were deter-

mined on the ration containing the 1% liver-extract powder. The hemorrhagic disease described above did not occur in those rats on the 10% germ-protein ration plus the 1% liver-extract powder. This was the reason for including the liver extract in the rations used in the work reported below. In the calculations of biological values the protein of the liver extract was included as part of the total protein of the ration.

The salt mixture used in the basal ration contained 14% calcium and 7.5% phosphorus. At a 4% level in the ration this salt mixture contributes 0.56% calcium and 0.30% phosphorus. The protein material used in the test diets contributed from 0.1% to 0.3% phosphorus.

The protein materials tested and their protein contents on a "natural" moisture basis were as follows:

Raw No. 1 wheat germ (N × 6.25)	28.5%
Processed wheat germ	28.5
Commercial casein	83.8
Dried lean beef muscle (overnite 50°C)	85.0
Skim milk powder (spray process)	34.5
Boiled, dry egg whites	86.0
Dried wheat gluten (55°C)	67.0
"Average Amer. Diet, Plant Sources"	11.7

The "average American diet, plant sources" used for some of the supplementation-power studies has the following composition:

	Percent by weight (dry basis)	Percent of total protein
Patent flour	44	49.0
Entire wheat	8	10.1
Yellow corn	23	19.1
Potatoes	20	18.6
Oatmeal	1.1	1.4
Rye	1.4	1.6
Rice	0.6	0.5
Barley	0.8	0.5
Buckwheat	1.1	1.2

None of the food materials were cooked. The potatoes were dried raw at room temperatures by means of a fan.

The animals: Four male albino rats, closely inbred and uniform, were used per group. They were housed individually in a room with the temperature controlled at 76°F. The rations were fed *ad libitum* and food consumptions recorded. The rats were weighed weekly during the four-week experimental period. The quality, or biological value, of the protein under test was determined from the average total weight gain in four weeks, the average food consumption during the corresponding period, and the protein (N × 6.25) contained in the mixed rations. The biological value is expressed as the gain in body weight per gram of protein consumed.

Results

Sole-source protein studies: The qualities of wheat-germ protein and of several animal proteins were determined by feeding them at various levels as the only proteins in the basal ration.

Table I shows the results obtained by feeding the various protein sources at about 5% levels. The liver-extract powder was omitted from the basal ration in this series. The variations within the groups are quite large, which is understandable when the low magnitude of body-weight gain and food-consumption figures are noted. However, there is little difference in the average biological values of the four

TABLE I

BIOLOGICAL VALUE OF WHEAT GERM PROTEIN AS COMPARED TO ANIMAL PROTEINS AS THE SOLE SOURCE OF PROTEIN AT A 5% LEVEL IN THE BASAL RATION

(Four rats per group. Time on experiment: four weeks. Starting weight of the rats 45 ± 4 g.)

Protein source	Protein in ration (N ×6.25)	H_2O in ration	Body weight gain, range and average		Food intake, range and average		Biological value of protein, range and average	
	%	%	g	g	g	g		
Wheat germ	4.70	2.0	12–25	19	197–235	211	1.40–2.88	2.12
Egg white	4.70	1.8	14–24	18	154–222	177	1.72–2.28	2.02
Skim milk	5.40	2.3	17–28	22	168–341	240	1.20–2.41	1.83
Casein +1% cystine	5.60	2.2	14–30	19	144–202	179	1.40–2.64	1.88

proteins tested at this low level. From these results it can be concluded that wheat-germ protein is at least as good in quality as are the animal proteins.

The results of feeding the protein sources at approximately a 10% protein level in the ration are shown in Table II. It appears that at this level small variations in the protein content of the ration have a marked effect on the apparent biological value. For example germ protein at 9.3% had a biological value of 2.87. This value fell to 2.41 when the protein content was increased to 11.7% of the ration. However, it is fully evident that the wheat-germ protein compared favorably with the various animal proteins tested.

The above results were obtained on suboptimal protein levels. The feeding of germ protein and other proteins at levels higher than 10% or 11% will not, of course, give significant figures on biological values, but will give further qualitative indications of the nutritional

TABLE II

BIOLOGICAL VALUE OF WHEAT GERM PROTEIN AS COMPARED TO ANIMAL PROTEINS AS THE SOLE SOURCE OF PROTEIN AT APPROXIMATELY 10% OF THE BASAL RATION

(Four rats per group. Time on experiment four weeks. Starting weight of the rats 45 ± 4 g.)

Protein source	Protein in ration (N ×6.25)	H_2O in ration	Body weight gain, range and average		Food intake, range and average		Biological value (growth/protein), range and average	
	%	%	g	g	g	g		
Wheat germ	9.3	3.8	54–64	59	218–225	222	2.60–3.15	2.87
	10.2	4.0	75–96	86	271–337	300	2.74–2.82	2.79
	11.4	5.6	90–102	97	311–357	338	2.43–2.57	2.52
	11.7	5.2	88–110	96	328–363	342	2.23–2.63	2.41
Casein	10.7	3.6	44–78	63	227–278	258	1.81–2.60	2.26
	11.4	4.7	73–84	79	275–314	296	2.21–2.40	2.34
Skim milk	10.7	3.0	81–117	100	262–374	327	2.70–2.92	2.85
Egg white	10.0	2.8	73–93	83	305–342	313	2.38–2.72	2.58

completeness of the protein of wheat germ. The results of higher-protein-level feeding are shown in Table III. A sample of wheat germ, processed in a manner which makes it a suitable food for human consumption and improves its keeping quality, was included in these tests and as can be seen from Table III the nutritional value of the protein was unimpaired.

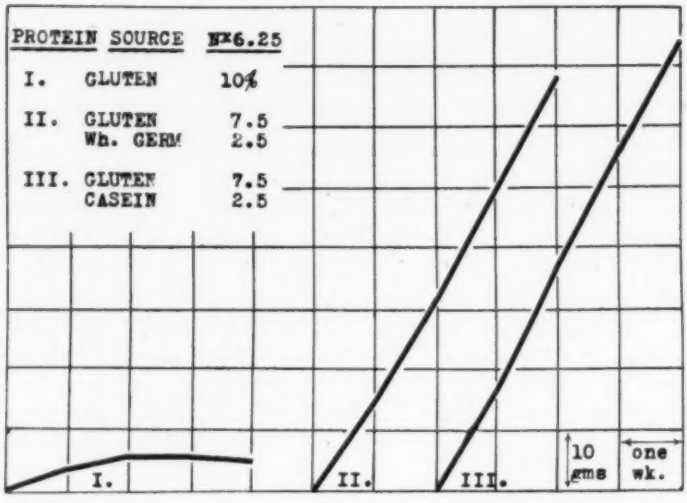
From the foregoing work it can be concluded that the protein of wheat germ as the sole protein in the diet is of as high a nutritional quality as animal proteins such as casein, skim milk powder, dried egg white, and beef muscle.

TABLE III

GROWTH OF RATS ON WHEAT GERM PROTEIN AS COMPARED WITH THE GROWTH ON CERTAIN ANIMAL PROTEINS AS THE SOLE PROTEIN SOURCE AT HIGHER LEVELS IN THE DIET

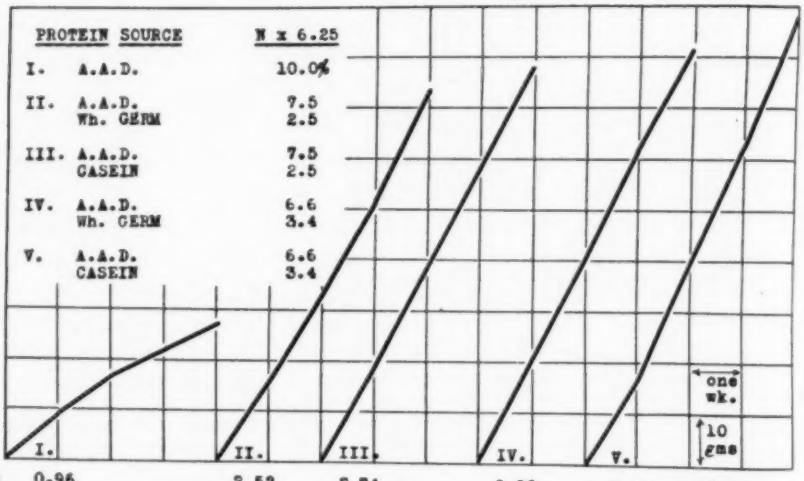
(Four rats per group. Time on experiment four weeks. Starting weight of the rats 45 ± 4 g.)

Protein source	Protein (N ×6.25)	H_2O in ration	Food intake, range and average		Body weight gain, range and average	
	%	%	g	g	g	g
Wheat germ, raw	18.3	8.7	352–366	360	126–132	128
Wheat germ, processed	17.8	8.1	360–415	387	136–140	138
Casein, commercial	18.0	4.2	324–395	358	121–148	131
Casein, commercial	15.4	4.6	352–359	355	108–118	114
Skim milk powder	15.2	3.9	339–368	354	116–129	122
Beef muscle (dried)	14.5	6.6	293–320	306	115–128	121
Beef muscle (dried)	35.0	8.7			120–136	128



GROWTH:	0.20	2.38	2.54	grams per day
BIOL. VALUE:	0.43	2.12	2.38	growth/gm. Protein

Fig. 1. The value of wheat-germ protein as compared with casein protein in the supplementation of gluten protein. Graphs represent growth rates of rats (4 per group) on diets containing 10% of protein.



GROWTH:	0.96	2.52	2.74	2.80	2.93	gms/day
BIOL. VALUE:	1.15	2.00	2.06	2.12	2.28	growth/gm. Protein

Fig. 2. The value of wheat-germ protein as compared with casein protein in the supplementation of proteins from the composite "plant sources of the American diet." Graphs represent growth rates of rats (4 per group) on diets containing 10% of protein. "A.A.D." represents "average American diet—plant portion."

The supplementing value of germ protein: Two experiments were carried out in which small amounts of wheat-germ protein were compared with the same amounts of casein as to their ability to improve the biological values of poor-quality vegetable proteins.

In the first experiment gluten was used as the basal protein source. The results are summarized in Figure 1. Gluten as the sole source of protein did not support growth. The substitution of one-fourth of the gluten protein with wheat-germ protein or casein protein resulted in marked growth responses of about equal degree for the two supplements, and a considerable increase in the biological values of the total proteins.

In the second experiment the composite "average American diet, plant sources" was used as the basal protein source. The results are shown in Figure 2. As the sole protein source the composite plant portions do support growth although not efficiently, the biological value being 1.15. Wheat-germ protein and casein protein are about equal in their supplementing values for this basal protein material.

Discussion

The results reported in this paper are, we believe, the first data indicating superior properties of wheat-germ protein for the growth of rats, as well as its equality to casein in supplementing poor-protein diets. An increased use of wheat germ in the American dietary would not only improve many of these diets (which are at present low in protein quality), but could also replace some of the animal proteins in the better diets. Furthermore wheat germ has high levels of thiamin and riboflavin, and is equal to whole wheat in nicotinic acid content. It is also an excellent source of many essential mineral elements such as copper, iron, and zinc.

What are the available quantities of wheat germ, which is a by-product of the milling industry? The milling yield is only about 0.5% of the wheat, although wheat actually contains close to 3.0% germ. At present the germ which is not separated as a distinct fraction during the milling process goes chiefly into the mill feed fractions—the bran, shorts, and midds. In 1941 about 15 million tons of wheat were milled into white flour in the United States. This indicates a potential germ production of at least 150 million pounds, with possibly three times this figure on the basis of higher yields. For comparison, there were 500 million pounds of dry skim milk powder produced in the United States during 1941 (Van Leer, 1942).

The objections to the use of wheat germ in the human dietary are its "green" or "feedy" taste and smell, and the fear of rancidity

due to its high fat content. These objections can be effectively removed by a number of procedures now available.

Summary

The protein of wheat germ has a high biological value as determined by the Osborne-Mendel rat-growth method. With protein levels of from 9.3% to 11.7% of the basal ration the biological value varied from 2.87 to 2.41. For comparison, the biological values of certain animal proteins fed at a 10% level were: commercial casein 2.30; dry skim milk, 2.85; boiled dry egg white, 2.58.

The biological value of the proteins derived from the composite plant sources of the "average American diet" was 1.15 at a 10% protein level in the diet of growing rats.

Wheat germ and casein proteins are equally effective as supplements to poor-protein diets. Rations in which the "average American diet, plant sources" furnished 7.5% protein and either wheat germ or casein furnished the remaining 2.5% showed total biological values of 2.00 and 2.06 respectively. Similarly, at 6.7% protein from the "average American diet" and 3.3% protein from wheat germ or casein, the biological values of the total proteins were 2.12 and 2.28, respectively.

At higher protein levels wheat germ as the sole protein in the diet promotes growth in young rats equal to that obtained on higher levels of casein, skim milk powder, or dry beef muscle.

Heat processing of wheat germ, adequate to make it suitable for human consumption, and to give it better keeping quality, has no effect on the biological value of the protein.

The yearly potential output of wheat germ in the United States has been calculated to have been at least 150 million pounds in 1941, assuming a milling yield of 0.5%. This is compared to the output of dry skim milk powder during 1941 of 500 million pounds.

It is suggested that wheat germ can be utilized in the human dietary and in nonruminant animal feeds as a supplemental protein of high biological value.

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THE EFFECT OF MILLROOM TEMPERATURE AND RELATIVE HUMIDITY ON EXPERIMENTAL FLOUR YIELDS AND FLOUR PROPERTIES [1]

E. G. BAYFIELD,[2] J. E. ANDERSON,[2]
and
W. F. GEDDES,[3] and F. C. HILDEBRAND [4]

(Read at the Annual Meeting, May 1942)

Atmospheric conditions have long been considered important factors in commercial milling. Many investigators have concluded that variations in these conditions have a pronounced effect upon the milling process and the character of the products. Thus Guthrie and Norris (1912) considered that flour moisture and total yields depended more on atmospheric conditions in the mill than upon the moisture content of the wheat. Miller (1924) stated that control of moisture in mill stocks is actually a problem of controlling the deficiency of moisture in the air in contact with these stocks. He considered that high air temperatures produced high evaporation losses and made proper conditioning of the air difficult.

Ferguson (1925) found that the commercial mill worked best when the conditioned air was kept between 65° and 70°F during the winter months and as cool during the summer as well water could make it. Conditioning the air increased the capacity of the mill during the summer months. Henkle (1928) observed during a 10-month operating period that the temperature ranged from 72° to 84°F, with an average relative humidity of 62%. He observed that evaporation from mill stocks during milling was twice as great during the summer as in the winter. Cooling by roll, purifier, and elevator suction was considered mainly due to evaporation. Pence (1933) presented data indicating that moisture is absorbed by some stocks when relative humidities in millrooms are maintained higher than 65%. He reported that variation in size of openings in silk bolting cloth due to changes in humidity in these experiments was so slight that little change in bolting capacity resulted.

Arnold (1937) considered that the most important part of air conditioning in the commercial mill was the removal of excessive heat. He maintained his roll floor at 78°F and 55% RH. Melvin (1922) preferred winter temperatures of 60°–70°F in the mill building and

[1] Contribution No. 87, Department of Milling Industry, Kansas Agricultural Experiment Station; Paper No. 2008, Scientific Journal Series, Minnesota Agricultural Experiment Station; Paper No. 44, Journal Series, General Mills, Inc., Research Laboratories.

[2] Head and Instructor, respectively, Department of Milling Industry, Kansas State College.

[3] Professor of Agricultural Biochemistry, University of Minnesota.

[4] Research Chemist, General Mills, Inc., Research Laboratories.

found that higher temperatures necessitated compensation through decreased rate of feed to the first break rolls.

Some millers prefer relatively low humidities in the millrooms. Thus Arnold (1937) quotes May as preferring 40% RH or lower. Robbins (1940) also considered 40% a desirable relative humidity for mill rooms. Vilm (1924) has pointed out that controlling the temperature of mill stocks and relative humidity *within* the roll stands was equally as important as controlling the atmospheric conditions in the mill rooms. He maintained that uniform temperature of stocks, obtained by correct roll settings, is important and cited temperature differences of as much as 44°F obtained by Herman (1924) when subjecting stocks to coarse or fine grinding. It seems evident that temperature changes of this magnitude, produced by slight differences in roll settings, are sufficient to influence milling results materially.

It is evident that any consideration of the effect of atmospheric conditions upon milling and mill products must include the possible effects of the interaction of many different factors—roll temperatures, rate of air movement, and moisture content of the stocks being among the more important. Anderson (1936) believed that more heat must be removed from the rolls and stocks by evaporation than by radiation; otherwise temperatures far above practical limits would be attained by the stocks and rolls. During milling the moisture content of various stocks tends toward equilibrium with the moisture of the surrounding environment, and variations in moisture content affect the yield of products materially. While Shollenberger (1921) found no apparent relationship between air temperature and yield of products, he did conclude that humidity was a minor factor of importance. He found no relationship between flour moisture and amount of added tempering water. This is opposed to the findings of Frank (1926), who found that moisture added to commercial mill mixes influenced the moisture content of the flour milled.

Considerable work has been reported upon the equilibrium relative humidities of wheat and flour, these studies indicating that with an increase in relative humidity the moisture content of the product may be expected to increase. Among others, Bailey (1920) observed that an 80% RH was required to maintain a 15% moisture content in flour. Coleman and Fellows (1925) measured the equilibrium relative humidity over hard winter wheat and found that 45% RH maintained 11.76% moisture in the wheat, 60% RH maintained 14.27% and 75% RH maintained 17.13%. Their observations showed that wheat responded readily to changes in humidity of the surrounding air, the rate of change depending upon the condition of exposure. Anderson (1937) found a change of about 5% in equilibrium humidity for each 0.9%

change in wheat moisture. Working with hard winter wheat he found 50% RH was needed to maintain 12% moisture in the wheat, 60% RH maintained 13.75% and 70% RH maintained 15.6% moisture in this lot of wheat. Recently Anker, Geddes, and Bailey (1942) have reported upon moisture changes of stored flour. Their results indicated clearly that flour moisture was influenced by both relative humidity and temperature of air in the storage chamber. They have reviewed the literature pertaining to flour moisture as influenced by humidity in storage.

Experimental milling, particularly with batch-type mills of the Allis type, differs drastically from continuous-flow commercial milling. In the former the mill stocks are exposed directly to the atmospheric conditions of the millroom to a varying degree, whereas in the latter the various stocks and flours are influenced only indirectly by the conditions in the mill building as the flour stocks are enclosed by the milling machines. It may be expected that the stocks within the milling machines are exposed to both warmer and more moist air than within the mill building for at least a major portion of the time during the milling operations, because moistened (tempered or conditioned) wheat is fed into the system continuously and heat is generated by the milling machines. Miller (1923) has pointed out the very different conditions encountered in experimental milling as compared to those in practical flour milling where heat generated by the rolls accumulates hour after hour. The heat generated by roll action and by other equipment in turn affects the amount of moisture in the air surrounding the equipment. These changes in turn affect milling to a varying degree. Markley and Bailey (1934) found that the diastatic activity of experimentally milled flour was reduced by increasing the relative humidity. On the other hand, Van Scoyk (1927) was unable to detect changes in ash, flour yield, flour color, or baking quality when the mill varied in humidity from 45% to 55% in one day or from 33% RH in December to 55% in July. The principal observable change was in flour moisture.

Reports of various investigations show that a relatively wide range of humidities has been used in experimental millrooms. Singh and Bailey (1940) used a relative humidity of 85%–90%; Geddes and West (1929), Geddes, Bergsteinnson, and Hadley (1933) used 70%; and McCluggage (1940), 50%. Less attention seems to have been given millroom temperatures, although Geddes and West (1929) concluded that variations in this factor had a significant effect on flour yield.

Micka and Vrana (1930) studied the influence of humidity and temperature upon sifting. They obtained the best results at 70°F and 70% RH. Ziegler (1940) studied the effect of several factors upon diastatic activity of flour. He believes that the higher maltose values

obtained from commercially milled flours are due to higher roll temperatures found in commercial plants.

Ziegler's conclusion is particularly interesting in view of roll temperature observations made by the senior author (unpublished) in grinding soft wheat. In this experiment temperatures of air and stock were taken automatically every two minutes immediately below the rolls in an Allis experimental mill operated in a room held at 80°F and 70% RH. It was found that the rolls required from 6 to 8 hours of milling time to attain an equilibrium condition. Approximately the same length of time was required for the rolls to reach room temperature after completion of the day's work. It was also found that lubricating the roll bearings with hard oil lowered the operating roll temperatures materially.

Hite (1940), from a study of data accumulated in a collaborative experiment, indicated that the use of controlled atmospheric conditions in the experimental millroom improves the reliability of flour yield determinations. Full atmospheric control (at 75°F and 65% RH) made possible the detection of differences in yield of one pound of wheat per barrel of flour. Bayfield, Bode, Hartsing, and Pettijohn (1940) and Bayfield, Bode, Hartsing, and Heizer (1940), using closely controlled millroom conditions (± 1°F and ± 2% RH) obtained definite differences in flour ash, viscosity, and baking results with soft wheats from two crop years by varying the atmospheric conditions in the experimental millroom.

Examination of work done by various investigators indicates the complexity of the problems concerning the atmospheric environment surrounding the flour particles during milling. Many seemingly contradictory statements are undoubtedly due to the action of one or more factors which have not been controlled during the course of the investigations. It is, however, evident that both atmospheric temperature and relative humidity influence the moisture content of flour. During milling the flour is exposed to the atmosphere in thin streams and therefore may be expected to respond rather rapidly to atmospheric changes. Furthermore, these atmospheric changes influence the milling equipment which in time may (if not completely compensated for by adjustment) produce changes in flour properties. Alsberg and Griffing (1925) concluded from their work that flour properties may be altered by mechanical treatment. If, as Miller (1941, p 466) maintains, the milling process influences flour quality, then a great deal of careful research will be required in order to catalogue the effects of the various factors which enter into milling.

The principal purposes of experimental milling are to find how a wheat may be expected to mill commercially without carrying out a

large-scale test on the commercial plant and to obtain a sample of flour which will be representative of flour which would be produced from the wheat if it were milled commercially. It is the consensus of opinion among practical millers that the milling operations do affect the qualities of the flour produced. If this be true then it is essential that flours produced in the laboratory should be milled under conditions and by methods which resemble the commercial operations as closely as possible. If this is not done then the analysis of the flour will not be made upon a product similar to that which will result from the commercial milling of the wheat. It is realized that experimental milling techniques available at present are unfortunately, at best, relatively crude approximations of the commercial process.

The studies reported at this time constitute a continuation of milling studies on hard red winter wheats which have been under way at Kansas State College for several years, and of the studies on soft winter wheats reported by Bayfield *et al* (1940). The present investigation had as its principal objectives: (1) to determine whether variation in millroom atmospheric conditions affects flour yields and flour properties; (2) to determine whether optimum atmospheric conditions for milling exist; (3) to determine whether different wheat varieties respond similarly to variations in the atmospheric conditions of the millroom.

Plan of Experiment

Two varieties of wheat were milled in duplicate on each of two mills at millroom temperatures of 70, 80, and 90°F and at 40, 50, 60, 70, 80, and 90% relative humidity at each temperature level, thus producing a total of 144 samples. The two varieties were milled in alternate order; because of the difficulty in altering millroom conditions the duplicate samples were milled on the same day.

Flour yield values were computed as percentage of straight grade flour. Granulation tests were made, by sifting, on all flours milled at 70°F and upon those milled at 80 and 90°F and 70% relative humidity.

Moisture, protein, ash, and diastatic activity determinations and baking tests were carried out in duplicate on all flours.

Materials and Methods

Bulk lots of high-grade 1939-crop pure Tenmarq and Kanred wheats grown in western Kansas were used in this investigation. Analytical data for these wheats are given in Table I. Sufficient wheat for the entire experiment was cleaned on a laboratory separator, dry-scoured and stored in large metal containers. A 2,000-gram subsample was withdrawn for each milling and tempered to 16% moisture by adding

TABLE I

ANALYSIS OF WHEAT

Variety	Test weight[1]	Moisture	Protein[2]	Ash[2]
	lbs	%	%	%
Tenmarq	62.0	10.4	15.7	1.54
Kanred	62.4	8.9	15.9	1.55

[1] Cleaned and scoured.
[2] 15% moisture basis.

water at approximately 65°F and allowing the sample to stand for 16 hours at approximately 90°F. The sample was again scoured and an additional 1% moisture added immediately prior to milling.

The wheats were milled on a Buhler mill (representative of the continuous-flow, enclosed, automatic type of experimental mill) and also on an Allis-Chalmers batch-type mill in which the stocks are handled manually. Specifications for these mills are shown in Table II.

Atmospheric conditions in the millroom were maintained within approximately ± 2°F and ± 3% RH by means of air circulated from a conditioning unit suspended near the ceiling. This unit heated or cooled, humidified or dehumidified the air automatically as required. The equipment was found somewhat deficient in capacity at the highest humidity levels, and supplementary humidification was supplied from a Carrier conditioner located in the same building. A continuous record of atmospheric conditions was obtained each day by means of a

TABLE II

SPECIFICATIONS FOR MILLS AND FLOWS USED IN EXPERIMENT

Mill	Allis	Buhler
Type of flow	discontinuous	continuous
Rate of feed	fixed	fixed
Breaks:		
Number used	4	3
Corrugations per inch	16 (all 4 breaks)	16, 21, 26 [1]
Spiral per foot	$\frac{3}{4}''$	$\frac{1}{2}''$ [1]
Type of corrugation	modified Dawson	saw tooth
Mode of operation	dull to dull	dull to dull
Differential	2.8 : 1	2 : 1
Roll adjustment	fixed [2]	fixed [3]
Reductions:		
Number used	9	3
Roll surface	sanded	Peerless cut
Differential	1.4 : 1	2 : 1
Roll adjustment	fixed [2]	fixed
Fast roll speed (rpm)	420	500
Sifting	fixed [4]	fixed

[1] Values only approximate—converted from metric system.
[2] Rolls set as accurately to constant spacing as possible.
[3] Set at beginning of day's operation and locked in place.
[4] Sifted for definite time interval, held constant throughout experiment.

recording wet and dry bulb thermometer, checked at intervals by a sling psychrometer.

About 18 hours before milling, the controls were brought into approximate adjustment for the conditions desired. The mills were started before actual milling operations were undertaken so that final adjustments could be made and the room conditions brought to equilibrium. Finally, a "warm-up" sample was milled on each mill simultaneously and then the actual experimental milling was undertaken. Both mills were in operation at the same time and all adjustments were made by the same operator.

At the end of each day the flours were rebolted through a 9 XX silk, thoroughly mixed mechanically and placed in tightly closed containers. Subsamples were taken for chemical analyses and for granulation tests, the balance being stored for about one month at room temperature and then held in cold storage until baked.

Granulation tests were made by mechanical sifting under controlled conditions using the following sieves: 14 XX, 18 S, 20 S, and 25 S, and the weight distribution determined.

Protein, ash, and diastatic activity determinations were made in duplicate as described in Cereal Laboratory Methods (4th ed., 1941). Flour yield, protein and ash values were corrected to a 15% moisture basis. All flours were baked in duplicate using the A. A. C. C. fermentation times and temperatures and a formula comprising 100 g flour (15% moisture basis), 6 g sugar, 1.5 g salt, 2 g yeast, 3 g shortening, 4 g dry milk solids, 3 mg potassium bromate, 0.25 g malt syrup, and sufficient distilled water for optimum absorption. The same mixing time was used for all samples of each variety.

Results

The data obtained have been submitted to analyses of variance. For these analyses, the mean values of duplicate analytical determinations and baking tests for each milling sample were taken; accordingly, the duplicate error is that between millings. To arrive at a value for experimental error, a comparison was made between the duplicate error and that of the combined second and third order interactions. If this interaction variance was significantly greater than the duplicate variance it was used as the error to test the significance of the various first order interactions. Any nonsignificant first-order interactions were then pooled with the higher-order interactions and the resulting value used as the error variance for testing the significance of the remaining variances. Such analyses were carried out on the data for each mill and for the combined results with both mills.

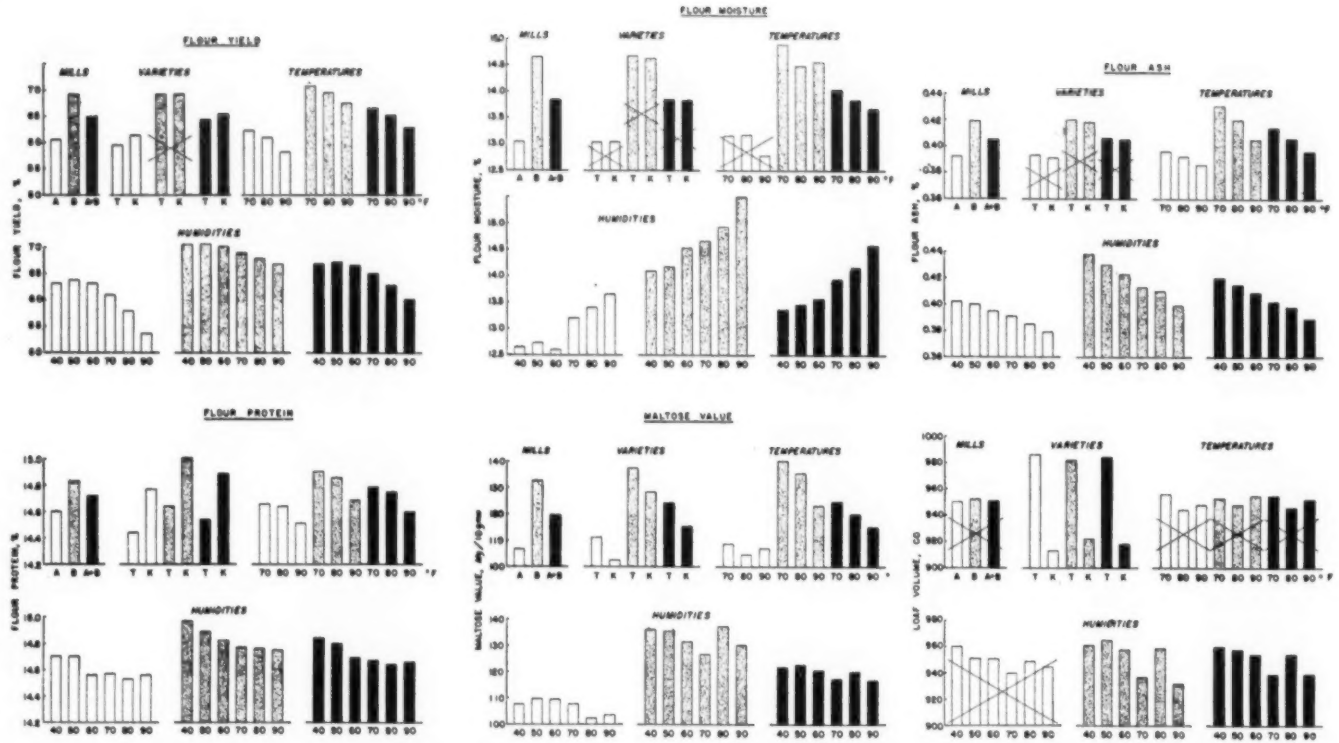

Fig. 1. Effect of mill type, wheat variety, temperature, and relative humidity of experimental millroom on flour yield and flour properties. Where a large X is drawn through the bars, the effect of the particular variable on the flour property under consideration is not statistically significant.

TABLE III

SIGNIFICANCE OF MAIN EFFECTS AND FIRST-ORDER INTERACTIONS[1]

Variance due to differences between	Yield			Granulation			Moisture			Ash		
	Al[2]	Bu[2]	Both	Al	Bu	Both	Al	Bu	Both	Al	Bu	Both
Mills			++			++			++			++
Varieties	++	−−	++	+	++	++	−−	−−	−−	−−	−−	−−
Temperature	++	++	++	−−	−−	+	−−	++	++	++	++	++
Humidities	++	++	++	+	++	++	+	++	++	++	++	++
Interactions												
M × V			++			−−			−−			−−
M × T			−−			−−			+			++
M × H			++			++			−−			+
V × T	−−	++	−−			−−	−−	−−	−−	−−	−−	−−
V × H	−−	−−	−−			−−	−−	−−	−−	−−	−−	−−
T × H	++	+	++				++	++	++	++	++	++

Variance due to differences between	Protein			Maltose value			Loaf volume		
	Al	Bu	Both	Al	Bu	Both	Al	Bu	Both
Mills			++			++			−−
Varieties	++	++	++	++	++	++	++	++	++
Temperature	++	++	++	++	++	++	−−	−−	−−
Humidities	++	++	++	++	++	++	−−	++	++
Interactions									
M × V			−−			−−			−−
M × T			−−			++			−−
M × H			−−			++			−−
V × T	−−	−−	−−	−−	−−	−−	−−	−−	−−
V × H	−−	−−	−−	−−	−−	−−	−−	−−	−−
T × H	−−	−−	−−	++	++	++	−−	−−	−−

[1] ++ denotes significance exceeding 1% point.
+ denotes significance exceeding 5% point.
−− denotes nonsignificance.
[2] Al = Allis; Bu = Buhler.

The results of these variance analyses are summarized in Table III, which shows the significance of the main effects and first-order interactions for flour yield and all flour properties studied. The general magnitude and direction of the effects of the principal variables on flour yield and each flour property, except granulation, are diagrammatically represented in Figure 1. For convenience and clarity the mean values and each significant interaction are tabulated separately for flour yield and each flour property.

Flour yield: The mean values for flour yield are shown in Table IV. The Buhler mill gave a much higher yield than the Allis under the conditions of this experiment. It should be mentioned, however, that all Allis mill yields obtained by using one pair of break rolls and one pair

TABLE IV

MEAN VALUES AND STANDARD ERRORS FOR FLOUR YIELD [1]

Variable	Mill		
	Allis	Buhler	Both
	%	%	%
Mills	**60.48**	**69.25**	(64.87)
Varieties			
Tenmarq	**59.52**	(69.22)	**64.37**
Kanred	**61.44**	(69.29)	**65.36**
Temperature			
70°F	**62.27**	**70.76**	**66.51**
80	**61.00**	**69.51**	**65.25**
90	**58.17**	**67.50**	**62.83**
Relative humidity			
40%	**63.16**	**70.69**	**66.93**
50	**63.84**	**70.75**	**67.30**
60	**63.16**	**70.17**	**66.67**
70	**61.00**	**69.11**	**65.06**
80	**57.99**	**67.89**	**62.94**
90	**53.71**	**66.90**	**60.30**
Standard error of single milling	1.47	1.33	1.40

[1] Where mean values are shown in **boldface,** the variable in question has a highly significant effect (exceeding 1% point); where values are shown in *italics* the effect is significant (exceeding 5% point); bracketed values indicate nonsignificance.

INTERACTION OF VARIETIES × TEMPERATURES—BUHLER MILL

Variety	Temperature			
	70°F	80°F	90°F	Mean
	%	%	%	%
Tenmarq	69.98	69.69	67.98	69.22
Kanred	71.53	69.32	67.01	69.29
Mean	70.76	69.51	67.50	69.25

Interaction significance exceeds 1% point.

INTERACTION OF TEMPERATURES × HUMIDITIES—ALLIS MILL

Relative humidity	Temperature			
	70°F	80°F	90°F	Mean
%	%	%	%	%
40	62.42	63.58	63.50	63.16
50	64.20	64.00	63.32	63.84
60	65.10	62.68	61.72	63.16
70	64.85	60.08	58.08	61.00
80	61.40	60.52	52.02	57.99
90	55.62	55.12	50.38	53.71
Mean	62.27	61.00	58.17	60.48

Interaction significance exceeds 1% point.

TABLE IV—(*Continued*)

INTERACTION OF TEMPERATURES × HUMIDITIES

BUHLER MILL

Relative humidity	Temperature			
	70°F	80°F	90°F	Mean
%	%	%	%	%
40	72.58	70.28	69.22	70.69
50	71.90	70.50	69.85	70.75
60	71.12	70.20	69.20	70.17
70	70.82	69.88	66.65	69.11
80	69.12	69.70	64.85	67.89
90	69.00	66.50	65.20	66.90
Mean	70.76	69 51	67.50	69.25

Interaction significance exceeds 5% point.

BOTH MILLS

Relative humidity	Temperature			
	70°F	80°F	90°F	Mean
%	%	%	%	%
40	67.50	66.92	66.36	66.93
50	68.05	67.25	66.59	67.30
60	68.11	66.44	65.46	66.67
70	67.84	64.98	62.36	65.06
80	65.26	65.11	58.44	62.94
90	62.31	60.81	57.79	60.30
Mean	66.51	65.25	62.83	64.87

Interaction significance exceeds 1% point.

of reduction rolls are at least 1.5% to 2.0% too low. This is due to incomplete clean-up of bran, sample spillage, and the presence of unreduced middlings found during rebolting which were not included in the flour weight. These middlings had apparently passed over the tops of the sieves during sifting and collected in the flour pan. Tenmarq wheat gave a lower yield on the Allis mill than did Kanred, whereas with the Buhler mill the yield values were virtually identical. This differential behavior is responsible for the significance of the mills × varieties interaction indicated in Table III. With each mill the yield decreased markedly with increasing millroom temperature and with increasing millroom humidity. The effect of temperature was consistent between mills but, as shown in Table IV, the effect of humidity was more pronounced with the Allis mill. This contrasting behavior presumably is to be ascribed to the fact that in the Allis mill the stocks are more exposed to the atmospheric conditions in the millroom than is the case with the Buhler. This effect is also reflected

in the increasing difference in yield between mills as the relative humidity is raised from 60% to 90%.

With each mill, the effect of humidity depended upon the temperature, becoming more pronounced as the temperature was increased; this is more noticeable in the instance of the Allis mill.

Flour granulation: Flour granulation tests were not made on all samples but only for the samples milled at 70°F for all relative humidities and at 70% relative humidity for all temperatures. Single siftings were made employing 14 XX, 18 S, 20 S, and 25 S on each of the duplicate millings for the above millroom conditions. The results were expressed as percentage overs on each sieve and the percentage through 25S. Inspection of the data indicated that millroom conditions affected only the percentages of the coarsest and finest fractions, the amount of each of the intermediate fractions being essentially constant. It follows, therefore, that the variations in the coarsest and finest fractions must be complementary since the total of all fractions equaled 100%. These assumptions were verified by calculation of the correlation between the overs on the 14 XX and the throughs passing the 25 S, a correlation coefficient of − .88 being obtained. Accordingly, the granulation of the flours could be adequately characterized by use of either of these measures. In this study the percentage flour passing the 25S was arbitrarily chosen for variance analysis, the results of which are shown in Tables III and V.

TABLE V

MEAN VALUES FOR FLOUR GRANULATION [1]

(Values as percent passing 25 S)

	Mill		
	Allis	Buhler	Both
	%	%	%
Mills	**38.4**	**48.0**	(43.2)
Varieties			
Tenmarq	*36.7*	**46.3**	**41.5**
Kanred	*40.2*	**49.8**	**45.0**
Temperatures			
70°F	(44.4)	(48.8)	*46.6*
80	(36.8)	(45.8)	*41.3*
90	(37.2)	(40.8)	*39.0*
Relative humidity			
40%	*38.0*	**44.4**	**41.2**
50	*36.2*	**45.0**	**40.6**
60	*33.1*	**47.0**	**40.1**
70	*44.4*	**48.8**	**46.6**
80	*43.3*	**50.2**	**46.8**
90	*35.6*	**52.6**	**44.1**

[1] Where mean values are shown in **boldface**, the variable in question has a highly significant effect (exceeding 1% point); where values are shown in *italics* the effect is significant (exceeding 5% point); bracketed values indicate nonsignificance.

TABLE VI

Mean Values and Standard Errors for Flour Moisture [1]

Variable	Mill		
	Allis	Buhler	Both
	%	%	%
Mills	**13.04**	**14.65**	(13.84)
Varieties			
Tenmarq	(13.03)	(14.68)	(13.85)
Kanred	(13.04)	(14.62)	(13.83)
Temperature			
70°F	(13.16)	**14.90**	**14.03**
80	(13.18)	**14.49**	**13.83**
90	(12.78)	**14.57**	**13.67**
Relative humidity			
40%	*12.64*	**14.09**	**13.36**
50	*12.73*	**14.17**	**13.45**
60	*12.59*	**14.53**	**13.56**
70	*13.20*	**14.67**	**13.94**
80	*13.40*	**14.93**	**14.16**
90	*13.66*	**15.50**	**14.58**
Standard error of single milling	0.369	0.179	0.289

[1] Where mean values are shown in **boldface,** the variable in question has a highly significant effect (exceeding 1% point); where values are shown in *italics* the effect is significant (exceeding 5% point); bracketed values indicate nonsignificance.

INTERACTION OF TEMPERATURES × HUMIDITIES—ALLIS MILL

Relative humidity	Temperature			
	70°F	80°F	90°F	Mean
%	%	%	%	%
40	12.98	12.71	12.24	12.64
50	12.41	12.92	12.85	12.73
60	12.69	12.81	12.28	12.59
70	13.71	13.00	12.90	13.20
80	14.25	13.56	12.38	13.40
90	12.90	14.06	14.01	13.66
Mean	13.16	13.18	12.78	13.04

Interaction significance exceeds 1% point.

BUHLER MILL

Relative humidity	Temperature			
	70°F	80°F	90°F	Mean
%	%	%	%	%
40	14.38	14.22	13.66	14.09
50	14.48	14.06	13.99	14.17
60	14.69	14.31	14.59	14.53
70	15.02	14.30	14.69	14.67
80	15.15	14.61	15.02	14.93
90	15.66	15.40	15.45	15.50
Mean	14.90	14.49	14.57	14.65

Interaction significance exceeds 1% point.

TABLE VI—(*Continued*)

INTERACTION OF TEMPERATURES × HUMIDITIES

BOTH MILLS

Relative humidity	Temperature			
	70°F	80°F	90°F	Mean
%	%	%	%	%
40	13.68	13.47	12.95	13.36
50	13.44	13.49	13.42	13.45
60	13.69	13.56	13.43	13.56
70	14.37	13.65	13.79	13.94
80	14.70	14.09	13.70	14.16
90	14.28	14.73	14.73	14.58
Mean	14.03	13.83	13.67	13.84

Interaction significance exceeds 1% point.

It is evident that a more finely granulated flour was produced on the Buhler than on the Allis mill; moreover with both mills, Kanred wheat gave a more finely ground flour. The magnitude of the difference was identical (3.5%) but because of the somewhat greater experimental error the significance of this difference was less in the case of the Allis mill data. Some difficulty was experienced in maintaining constant roll settings with the Allis mill, which may account for the relatively greater variability in granulation data for the flours produced by this mill.

When the data for both mills were considered together, increasing temperature tended to produce a more coarsely granulated flour; for the individual mills similar trends were noted but the differences were not sufficiently great to offset the loss in precision caused by the reduction in the amount of data available for analysis. The effect of humidity on granulation was not consistent for the two mills. In the case of the Allis mill, the most finely granulated flour was produced at 70% relative humidity with a decrease in the percentage of fines as the humidity was varied in either direction from this level. In contrast, in the case of the Buhler mill, flour fineness increased progressively with increasing humidity.

Flour moisture: Referring to Table VI, it will be seen that the Buhler mill produced flours of much higher average moisture content than the Allis; this difference may be accounted for by the relatively greater exposure of the stocks to the atmosphere in the case of the Allis mill. In no case was there any significant difference in flour moisture between varieties.

Taking the results for the two mills combined, flour moisture decreased with increasing millroom temperature, but the effect was not

TABLE VII

MEAN VALUES AND STANDARD ERRORS FOR FLOUR ASH [1]

Variable	Mills		
	Allis	Buhler	Both
	%	%	%
Mills	**0.392**	**0.419**	(0.405)
Varieties			
Tenmarq	(0.393)	(0.420)	(0.406)
Kanred	(0.391)	(0.418)	(0.405)
Temperature			
70°F	**0.396**	**0.431**	**0.414**
80	**0.392**	**0.420**	**0.406**
90	**0.386**	**0.405**	**0.396**
Relative humidity			
40%	**0.402**	**0.438**	**0.420**
50	**0.400**	**0.430**	**0.415**
60	**0.395**	**0.423**	**0.409**
70	**0.391**	**0.413**	**0.402**
80	**0.385**	**0.410**	**0.398**
90	**0.379**	**0.399**	**0.389**
Standard error of single milling	0.0044	0.0047	0.0045

[1] Where mean values are shown in **boldface,** the variable in question has a highly significant effect (exceeding 1% point); where values are shown in *italics* the effect is significant (exceeding 5% point); bracketed values indicate nonsignificance.

INTERACTION OF TEMPERATURES × HUMIDITIES—ALLIS MILL

Relative humidity	Temperature			
	70°F	80°F	90°F	Mean
%	%	%	%	%
40	0.412	0.402	0.392	0.402
50	.405	.404	.392	.400
60	.392	.400	.392	.395
70	.399	.389	.384	.391
80	.380	.384	.390	.385
90	.390	.376	.370	.379
Mean	.396	.392	.386	.392

Interaction significance exceeds 1% point.

BUHLER MILL

Relative humidity	Temperature			
	70°F	80°F	90°F	Mean
%	%	%	%	%
40	0.462	0.433	0.419	0.438
50	.440	.433	.418	.430
60	.438	.426	.404	.423
70	.416	.420	.402	.413
80	.416	.416	.399	.410
90	.416	.391	.390	.399
Mean	.431	.420	.405	.419

Interaction significance exceeds 1% point.

TABLE VII—(*Continued*)

INTERACTION OF TEMPERATURES × HUMIDITIES—BOTH MILLS

Relative humidity	Temperature			
	70°F	80°F	90°F	Mean
%	%	%	%	%
40	0.437	0.418	0.406	0.420
50	.423	.418	.405	.415
60	.415	.413	.398	.409
70	.408	.405	.393	.402
80	.398	.400	.394	.398
90	.403	.383	.380	.389
Mean	.414	.406	.396	.405

Interaction significance exceeds 1% point.

consistent for the individual mills. In the Allis mill, the differences due to temperature were not significant. It is believed that this behavior may be accounted for by the high experimental error for flour moisture with this mill, due to trouble experienced with heating of the rolls noted on some samples. In the instance of the Buhler mill, flour moisture decreased as the millroom temperature was raised from 70° to 80°F and then increased slightly with a further increase in temperature to 90°F. The difference in temperature response between the two mills is also indicated by the significance of the mills × temperatures interaction.

As would be expected, increasing relative humidity is reflected in increased flour moisture. The lesser significance of this effect in the case of the Allis mill is due to the higher experimental error mentioned above. The essential consistency of the humidity response with the two mills is indicated by the nonsignificance of the mills × humidities interaction.

Highly significant temperatures × humidities interactions, found for each mill, are also shown in Table VI. As would be expected the effect of variations in humidity became more pronounced with increasing temperature.

Flour ash: From the data in Table VII, it will be seen that the Buhler mill produced flours of significantly higher average ash content than the Allis mill, which is in accord with the higher flour yield obtained with the Buhler mill. The two varieties tested did not differ in the ash content of the flours produced therefrom.

Flour ash, with each mill, decreased with increasing temperature and with increasing relative humidity. The magnitude of these effects was greater with the Buhler mill, thus giving rise to significant mills × temperatures and mills × humidities interactions. With each mill, the decrease in flour ash with increasing millroom temperature was

greater at the lower than at the higher humidities; this is responsible for significant temperatures × humidities interactions.

Flour protein: Table VIII gives the effects of the several variables upon flour protein. Significantly higher protein values were obtained with the Buhler mill, with Kanred wheat, with lower temperatures and with lower humidities. All effects were consistent throughout, as indicated by the absence of significant interactions.

TABLE VIII

MEAN VALUES AND STANDARD ERRORS FOR FLOUR PROTEIN [1]

Variable	Mill		
	Allis	Buhler	Both
	%	%	%
Mills	**14.60**	**14.83**	(14.72)
Varieties			
Tenmarq	**14.44**	**14.64**	**14.54**
Kanred	**14.77**	**15.01**	**14.89**
Temperature			
70°F	**14.66**	**14.93**	**14.79**
80	**14.64**	**14.86**	**14.75**
90	**14.51**	**14.69**	**14.60**
Relative humidity			
40%	**14.70**	**14.97**	**14.84**
50	**14.70**	**14.89**	**14.80**
60	**14.56**	**14.82**	**14.69**
70	**14.57**	**14.77**	**14.67**
80	**14.53**	**14.76**	**14.64**
90	**14.56**	**14.75**	**14.66**
Standard error of single milling	0.103	0.135	0.120

[1] Where mean values are shown in **boldface,** the variable in question has a highly significant effect (exceeding 1% point); where values are shown in *italics* the effect is significant (exceeding 5% point); bracketed values indicate nonsignificance.

Maltose value: Considering the maltose values tabulated in Table IX, it is apparent that the Buhler mill flours exhibit much higher values than comparable Allis mill flours, that Tenmarq wheat gives flour of higher activity than Kanred, and that increase in millroom temperature brings about a lowered diastatic activity. The last-named effect is not consistent for both mills, the action of temperature being more pronounced in the case of the Buhler mill.

While variations in millroom humidity have a statistically significant effect on maltose value, no consistent trend is apparent with either mill. Thus for the Allis mill the highest values were obtained at 50% and 60% relative humidity and the lowest at 80% and 90% whereas, for the Buhler mill, high values were obtained at 40, 50, and 80% relative humidity and the lowest value at 70%. For both mills combined, the lower relative humidities gave slightly higher maltose values than those for 70, 80, and 90% relative humidity. Significant inter-

TABLE IX

MEAN VALUES AND STANDARD ERRORS FOR MALTOSE VALUE [1]

Variable	Mill		
	Allis	Buhler	Both
	mg/10 g	*mg/10 g*	*mg/10 g*
Mills	**106.7**	**132.8**	(119.8)
Varieties			
Tenmarq	**111.0**	**137.4**	**124.2**
Kanred	**102.4**	**128.3**	**115.3**
Temperature			
70°F	**108.8**	**140.1**	**124.5**
80	**104.4**	**135.3**	**119.8**
90	**106.9**	**123.1**	**115.0**
Relative humidity			
40%	**107.5**	**136.1**	**121.8**
50	**109.7**	**135.5**	**122.6**
60	**109.4**	**131.5**	**120.5**
70	**107.7**	**126.6**	**117.2**
80	**102.2**	**137.2**	**119.8**
90	**103.6**	**130.1**	**116.8**
Standard error of single milling	4.48	4.35	4.42

[1] Where mean values are shown in **boldface,** the variable in question has a highly significant effect (exceeding 1% point); where values are shown in *italics* the effect is significant (exceeding 5% point); bracketed values indicate nonsignificance.

INTERACTION OF TEMPERATURES × HUMIDITIES—ALLIS MILL

Relative humidity	Temperature			
	70°F	80°F	90°F	Mean
%	*mg/10 g*	*mg/10 g*	*mg/10 g*	*mg/10 g*
40	108.2	107.5	106.8	107.5
50	113.0	106.2	110.0	109.7
60	107.0	106.0	115.2	109.4
70	109.5	102.5	111.2	107.7
80	102.0	102.2	102.5	102.2
90	113.2	101.8	95.8	103.6
Mean	108.8	104.4	106.9	106.7

Interaction significance exceeds 1% point.

BUHLER MILL

Relative humidity	Temperature			
	70°F	80°F	90°F	Mean
%	*mg/10 g*	*mg/10 g*	*mg/10 g*	*mg/10 g*
40	141.8	140.0	126.5	136.1
50	140.8	140.8	125.0	135.5
60	144.2	129.2	121.0	131.5
70	131.8	127.2	120.8	126.6
80	146.2	143.8	121.8	137.2
90	136.0	130.8	123.5	130.1
Mean	140.1	135.3	123.1	132.8

Interaction significance exceeds 1% point.

TABLE IX—(*Continued*)

INTERACTION OF TEMPERATURE × HUMIDITIES—BOTH MILLS

Relative humidity	Temperature			
	70°F	80°F	90°F	Mean
%	*mg/10 g*	*mg/10 g*	*mg/10 g*	*mg/10 g*
40	125.0	123.8	116.7	121.8
50	126.9	123.5	117.5	122.6
60	125.6	117.6	118.1	120.5
70	120.6	114.9	116.0	117.2
80	124.1	123.0	112.1	119.8
90	124.6	116.2	109.6	116.8
Mean	124.5	119.8	115.0	119.8

Interaction significance exceeds 1% point.

actions for temperatures × humidities were found for each mill and for both mills combined. The nature of the inconsistencies leading to these interactions was not the same for both mills, since a significant triple interaction (mills × temperatures × humidities) was found. Accordingly, it is not possible to generalize on the combined effect of temperature and humidity on maltose value.

Loaf volume: It is of particular interest to note that whereas with most flour properties there has been a significant effect of all variables, in the case of loaf volume the only factors exerting a significant effect are variety and relative humidity, as shown by the data of Table X.

TABLE X

MEAN VALUES AND STANDARD ERRORS FOR LOAF VOLUME [1]

Variable	Mill		
	Allis	Buhler	Both
	cc	*cc*	*cc*
Mills	(950)	(952)	(951)
Varieties			
Tenmarq	**986**	**982**	**984**
Kanred	**913**	**922**	**918**
Temperature			
70°F	(956)	(953)	(955)
80	(944)	(948)	(946)
90	(948)	(955)	(952)
Relative humidity			
40%	(960)	**961**	**960**
50	(951)	**965**	**958**
60	(951)	**958**	**954**
70	(940)	**937**	**939**
80	(949)	**959**	**954**
90	(945)	**932**	**939**
Standard error of single milling	21.5	23.9	22.7

[1] Where mean values are shown in **boldface,** the variable in question has a highly significant effect (exceeding 1% point); where values are shown in *italics* the effect is significant (exceeding 5% point); bracketed values indicate nonsignificance.

Whether this may or may not be due to the baking formula used is not known. In all cases the loaf volumes for Tenmarq flours were much higher than those for flours milled from Kanred wheat. With the Buhler mill and with both mills combined the values for 70% and 90% relative humidity were extremely low in comparison with the values for other humidity levels. While a similar tendency was noted with the Allis mill, the effect of humidity was not significant. The existence of a similar trend is supported by the absence of a significant mills × humidities interaction.

Discussion

These studies clearly demonstrate that variations in millroom conditions influence flour yield and all flour properties investigated. It is of interest to note that the effect of these variations gives rise to fewer statistically significant differences in the instances of loaf volume than in the case of other flour properties or flour yield. This may, however, be a reflection of lower precision of the baking test or of the use of a commercial-type formula which possibly tends to obscure differences in flour characteristics. The variation in humidity employed in these experiments brought about greater differences than temperature in all flour properties except protein content, in which the effect was the same, and maltose value in which temperature had much the greater influence. It necessarily follows from these observations that the replicability of experimental milling results will be improved by controlling the atmospheric conditions in the millroom.

The question next arises as to whether an optimum set of millroom conditions exists. In defining such optimum conditions one must of necessity consider the purpose of conducting the experimental milling test. Such tests are carried out either to provide representative samples from different lots of wheat for further quality tests (such as ash, color, and baking strength) or they may be used to characterize the milling behavior of the wheats.

In considering the first of these purposes it would be desirable to select atmospheric conditions which would tend to produce flours as nearly as possible identical in properties with those which would be obtained by commercial-scale milling. The use of such conditions would minimize the need for translation of results. In the absence of information regarding the characteristics of flours which could be milled commercially from the wheats used in this investigation, no recommendations can be made as to specific experimental millroom conditions. However, it is worthy of note that the choice of any particular temperature and relative humidity will not significantly affect the differentiation between these two wheat samples in regard to the properties of the

flours produced therefrom. This is demonstrated by the absence of significant interaction of variety with temperature or humidity for any of the flour properties studied.

With reference to the use of the milling test to characterize the milling behavior of different wheats it should be noted that there is only one significant interaction involving variety and millroom conditions for flour yield, namely, that between variety and temperature with the Buhler mill.

Summary

Two varieties of hard red winter wheat (Tenmarq and Kanred) were milled in duplicate on both Allis and Buhler mills at millroom temperatures of 70, 80, and 90°F and relative humidities of 40, 50, 60, 70, 80, and 90%, thus producing 144 flours. Determinations were made of flour yield, granulation, moisture, ash, protein, maltose value, and loaf volume and the data submitted to statistical analysis.

Flour yield decreased with increasing temperature and humidity, the effect of humidity being greater at the higher temperatures. Yields from the Allis mill were lower and affected more by humidity variations than those from the Buhler mill. The effect of temperature was greater in the instance of Kanred wheat.

Flour granulation, determined with only a limited number of samples, tended to become coarser with increasing temperature. Increasing humidity increased flour fineness in the case of the Buhler, whereas with the Allis mill the most finely granulated flour was obtained at 70% relative humidity. A more finely granulated flour was produced by the Buhler than the Allis mill and by Kanred in contrast to Tenmarq wheat.

Flour moisture increased with decreasing temperature and with increasing humidity. The Buhler mill produced higher-moisture flour than the Allis.

Flour ash increased with decreasing temperature and relative humidity. The Buhler mill gave higher-ash flour than the Allis.

Flour protein responded to variations in temperatures, humidities and mills in the same manner as flour ash.

Maltose value increased with decreasing temperature, while the effect of humidity was relatively slight and not consistent. Buhler-milled flours gave much higher values than Allis-milled flours.

Loaf volume was significantly affected by relative humidity, the lowest values being obtained at 70% and 90%. Temperature and mills were without effect.

Replicability of experimental milling results should be improved by control of millroom atmospheric conditions. No indication of the

existence of optimum millroom conditions for differentiation between wheat samples is shown by these experiments, although the possibility is not excluded that further studies may lead to the formulation of optimum atmospheric conditions.

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MICRO TESTS OF ALIMENTARY PASTES. I. APPARATUS AND METHOD [1]

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Cereal chemists, in providing a service for plant breeders, have continuously striven to reduce the amount of grain required for preliminary tests of the quality of new varieties. Durum wheat is no exception; Fifield, Smith, and Hayes (1937) have described a micro technique for assessing macaroni-making quality. The method requires only 30 g of semolina and involves the preparation of pressed disks of alimentary paste and subsequent study of their color characteristics.

In this laboratory difficulty was experienced in obtaining reproducible results by this method, but attempts to devise more satisfactory techniques of other sorts proved unfruitful. It therefore seemed advisable to concentrate on the improvement of the disk

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method. Considerable stimulus was given to this work when it was observed that the opacity of the disks was closely related to their color characteristics and very sensitive to changes in processing conditions. A photoelectric method of measuring opacity was therefore developed, and with this precise and objective tool rapid progress was made in elucidating the factors that affect the reproducibility of the test. A comprehensive study of the effect of processing conditions on paste properties was then undertaken and will be described in a second paper. The present paper is introductory and serves to put on record descriptions of the apparatus and methods now used in the Board of Grain Commissioners' Laboratory for micro tests of alimentary paste.

Preparation of Disks

The original apparatus and method used in preparing the disks have been described in detail by Fifield, Smith, and Hayes (1937). Accordingly, the following description is confined mainly to the modifications used in this laboratory.

Mixer and mixing: A photograph of the mechanically driven, thermostatically controlled mixer is shown in Figure 1. The sheeting rolls, described in the next subsection, also appear on the right in this photograph. The mixer is similar to that of Fifield, with rotating blades on a horizontal metal shaft. The mixer has a maximum capacity of 50 g of semolina, but as little as 30 g can be used in routine studies. Since the temperature and speed of mixing are important, improvements were made in the original model by introducing temperature control and a mechanical drive. Temperature is controlled at 30°C by surrounding the mixer with a water bath (9.0 × 7.5 × 5.0 inches) provided with a motor-driven stirrer and a thermoregulator. The six brass disks on the top of the bath (Fig. 1) are the lids of removable cylindrical chambers (4 inches deep, 1.5 inches diameter) in which samples of semolina are conditioned prior to mixing. An 0.12 hp reversible electric motor with reduction gears turning 57 rpm was available in the laboratory and is used to drive the mixer. A reversing switch is also used and can be seen in the photograph.

With the drive reversed three times during the mixing period, a mixing time of between 30 and 100 seconds has been found satisfactory for doughs falling within a reasonable working range of consistency (*i.e.*, absorption 28 to 32%). Experiments showed that 40 seconds of mixing was roughly equivalent to 4 minutes of mixing in the larger mixer described by Binnington and Geddes (1936), and 40 seconds was therefore tentatively adopted as a standard mixing time.

On the basis of such data as are available, the writers believe that it is feasible to use a constant absorption of 30%. The alternative

method of adjusting the absorption for different semolinas so as to obtain constant consistency increases the amount of semolina required for the micro test, since portions must be used for preliminary determination of the correct absorption. Consistency is not as important in the micro test as in making macaroni. In the latter process the pressure to which the dough is subjected in forcing it through the die depends on the consistency, so that this must be held constant in order to subject all

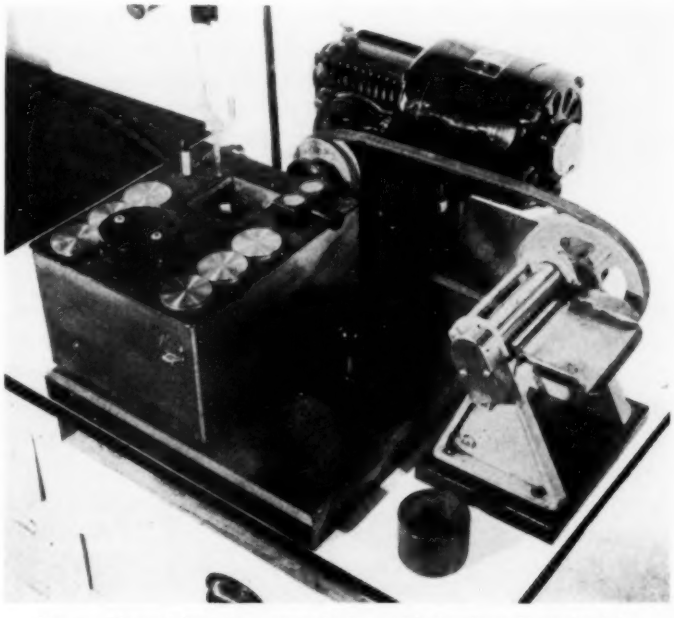

Fig. 1. Motor driven thermostated mixer, with sheeting rolls at the right. The top of the mixer can be seen below the burette.

doughs to equal pressures. In the micro test the pressure applied is independent of the consistency of the dough. For this reason, and because the interaction between semolinas and absorption appears to be relatively small, it seems feasible to use a constant absorption in the micro test.

Sheeting: No rest period is used in this laboratory as this step is not readily controlled and appears to have no compensating advantages. Immediately after mixing, the dough is put through a pair of smooth sheeting rolls (Fig. 1), 4 inches long by 1 inch in diameter and $\frac{1}{8}$ inch apart, operated at room temperature (about 24°C). These rolls are similar to those described by Fifield *et al* except that they are motor driven at 45 rpm. A uniform rate of sheeting facilitates the preparation of disks of uniform thickness. Hand-operated rolls are satisfactory

provided they are turned at a uniform speed, but a mechanical drive has been found convenient.

The dough is sheeted, folded end to end and sheeted again in the same direction, putting the joined ends through first so that the crease is rolled last. After sheeting three times, the folded dough is turned through an angle of 90° so that the sides enter the rolls first. This turn is made after every third sheeting. In addition, the dough is turned over after each sheeting so that alternate sides of the sheet are folded in. The process is repeated until the dough has been sheeted and folded 15 times. Experiments with various methods of folding indicate that the method described above gives the most uniform sheets of dough.

Pressing: Two disks are cut from the sheet of dough with a circular cutter $2\frac{3}{16}$ inches in diameter. Three celluloid disks of the same size are dipped in a benzene solution of Parawax (70 g per liter) and allowed to dry for 20 minutes at room temperature. The two paste disks and the three celluloid disks are then made into a double-decker sandwich, placed in a press bowl ($2\frac{3}{16}$ inches internal diameter) and pressed in a Carver laboratory press. It should be noted that the press must be provided with a low-range gauge, and must be free from leaks so that the desired pressure can be maintained with minimum periodic pumping. Pressing for 7 minutes at a gauge pressure of 1,000 pounds per square inch (about 750 pounds per square inch on the paste disks) normally produces disks having an internal structure similar to that of tubular macaroni, as judged by visual inspection. After pressing, the assembly of disks is removed and the disks separated, particular care being taken not to mar the surfaces of the paste.

The principal difference between this pressing technique and that of Fifield *et al* is that wax-coated celluloid rather than cellophane disks are used to encase the paste. While the use of wax represents a radical departure from ordinary technique, this modification facilitates the preparation of disks having smooth uniform surfaces, and these are absolutely essential for the accurate determination of the absorption coefficient described later.

Drying: The method used is again a slight modification of that of Fifield *et al.* The disk is laid between 4-inch squares of bond paper, backed with three layers of blotting paper and this assembly is then clamped between 4-inch squares of heavy wire gauze by means of large "Bulldog" clips. A series of these assemblies can then be conveniently strung on a metal rod for drying. After drying for two days in a room controlled at 26° ± 2°C the disks are removed and their quality characteristics measured.

For the most precise work, and particularly when strictly comparable results are required in experiments made at different times of the year, it appears that both temperature and humidity should be controlled during drying. However, experiments have shown that considerable changes in temperature and humidity have a relatively small effect on the quality of the finished disks. Accordingly, for most practical purposes, drying can be carried out under room conditions.

Measurement of the Optical Coefficient of Absorption, or Degree of Opacity

The idea of developing an objective photometric measurement of opacity occurred to one of us (R. L. C.) during early studies of the effect of processing methods on the color of disks. It was observed that if two disks made from the same semolina differed in opacity, then the

Fig. 2. General view of photometer.

more opaque disk appeared less yellow. Since both contained the same quantity of pigment it was believed that the more translucent looked yellower because the light penetrated more deeply and was thus reflected from additional and deeper layers of pigment molecules.

It thus appeared that the measurement of opacity might prove a useful supplement to the commonly used measurement of disk color, by matching against Wallace and Tiernan disks (Baker, 1939). This latter process is tedious, subjective, lacking in precision, and subject to an appreciable personal error. On the other hand, it seemed clear that a photometric measurement of the absorption coefficient would be objective, precise, and reproducible. These expectations have been fully

justified since the new method gives exactly the same results in the hands of different technicians.

Photometer: A photograph of the photometer used for measuring the absorption coefficients of the disks appears in Figure 2. It consists essentially of a light source and a photronic cell, between which there is inserted a turn-table holding the paste disks. The light and turn-table can be seen on top of the case. The photronic cell, wiring, rheostat, switches, and the motor which drives the turn-table are inside the case. A voltmeter, mounted on the end of the case, is used to aid in making rough adjustments of the light intensity.

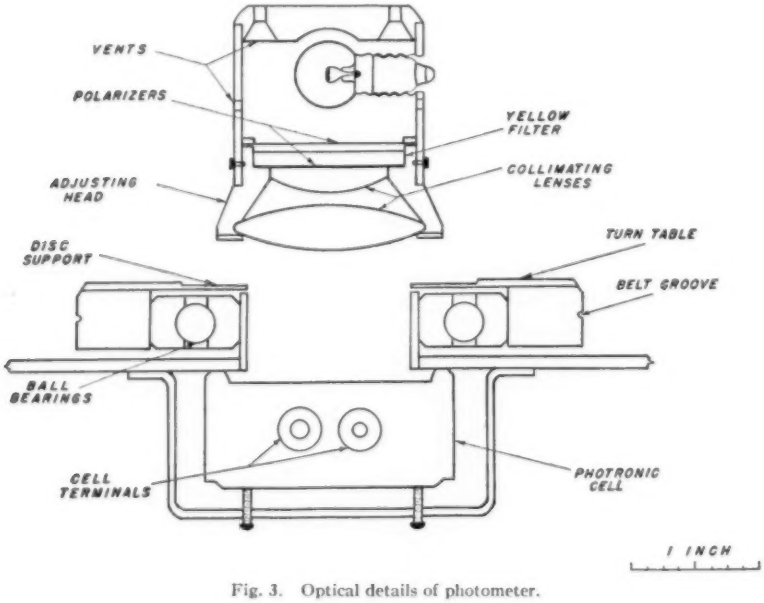

Fig. 3. Optical details of photometer.

As the photometer is of simple design, and as there are no special virtues in its dimensions, a short description of the main features will serve as an adequate guide to anyone interested in constructing one. The essential details are shown in Figure 3. The light source consists of a 9-volt flashlight bulb mounted in a suitable ventilated brass housing. The interior of the housing has a dull black finish which eliminates reflection from the walls. Two lenses are used to produce a parallel beam of light, thus minimizing the effect of small changes in the distance between the light source and the paste disk. Although the lamp operates at fixed voltage by means of batteries, it was necessary to introduce a fine adjustment for light intensity. This consists of two sheets of Polaroid ("J" film), the lower of which is mounted in an adjusting

head. It will be apparent that this provides a control of light intensity without varying the area of the disk subjected to illumination as would be the case with the use of an iris diaphram. A yellow filter (Wratten K2) is also included in the assembly. The filter eliminates those wave lengths most strongly absorbed by the pigment in the paste and thus serves to make the measurement of the opacity relatively independent of the pigment concentration in the paste.

The paste disks are held on an electrically driven turn-table mounted on ball bearings and rotating at 200 rpm. As a disk is not uniformly opaque and the surface of a photronic cell varies in sensitivity from point to point, a turn-table is required in order that the disks may be scanned by each sector of the cell. The inequalities of both cell and disk are thus integrated and an accurate and reproducible measurement of the transmitted light is obtained.

The voltage generated by the photocell is measured with a potentiometer in the usual manner.

A disk having permanent opacity characteristics similar to those of an average paste disk, was required for standardizing the instrument. This was prepared by combining a slightly exposed photographic film and two sheets of photographic glass in a brass assembly of the same diameter as the paste disks ($2\frac{3}{16}$ inches). After calibrating the photronic cell for various light intensities, the exact percentage transmission of the standard disk was determined and found to be 19.4%.

In making measurements the standard disk is placed on the turn-table before each reading and the light intensity is checked (and adjusted by means of the Polaroid, if necessary) by noting the voltage generated by the photronic cell. After removing the standard, the paste disk is placed on the turn-table and the voltage generated by the cell is determined.

Variations of about 5% occur frequently in the thickness of different disks and variations of as much as 10% occur occasionally. So far as can be determined, these differences in the thickness of different disks result from variations in the thickness of the sheeted dough. Stiff doughs and rapid sheeting produce thicker disks and in spite of careful attention to sheeting technique, some variations in thickness occur between different parts of the same sheet of dough. As the thickness of the disk must be taken into account in calculating the absorption coefficient, it is taken as the mean of nine micrometer readings made to the nearest 0.001 cm.

Calculation of absorption coefficient: The absorption coefficient, which is a measure of the loss of intensity which results when light passes through a unit thickness of paste, is calculated from an equation based

on Lambert's law. This equation is:

$$-K = \frac{1}{t} \log_e CP_t, \tag{1}$$

where K is the absorption coefficient, t is the thickness of the disk, C is a factor which corrects for loss of light by reflection at the air-solid interfaces, and P_t is the emerging fraction of the light which enters the paste.

P_t is determined by comparing the paste disk with the standard disk described above. This is done by determining the relative voltages generated in the photocell and making the necessary small corrections for variations in cell sensitivity at various intensities of illumination. We then have:

$$\frac{P_t}{P_s} = \frac{V_t}{V_s} \quad \text{or} \quad P_t = P_s \frac{V_t}{V_s}, \tag{2}$$

where P_s is the fraction of light transmitted by the standard disk and V_t and V_s are the corrected voltages generated by the light after passing through the test and standard disks respectively.

The equation may therefore be rewritten:

$$-K = \frac{1}{t} \log_e CP_s \frac{V_t}{V_s}. \tag{3}$$

As previously noted, the value of P_s was determined experimentally and was found to be 0.194. V_s depends upon the type of photocell used, the intensity of the light source, as well as the voltage input of the potentiometer. It was arbitrarily chosen as 500 units. The equation is thus:

$$-K = \frac{1}{t} \log_e C \frac{.194}{500} V_t. \tag{4}$$

In dealing with a homogeneous solid such as glass the value of the factor C, which corrects for reflection losses, can be calculated from the index of refraction for glass in accordance with Fresnel's formula:

$$\frac{1}{C} = \left[1 - \left(\frac{n-1}{n+1} \right)^2 \right]^x, \tag{5}$$

where n is the index of refraction of glass and x is the number of air-glass interfaces, which will be 2 for a plate of glass.

In dealing with alimentary paste the calculation of C is complicated by two factors. First, the index of refraction of the paste is not known and would be difficult to determine experimentally. Secondly, in paste disks there is a concentration of microscopic cracks and bubbles

near each surface *over and above* the normal distribution of these throughout the body of the paste. These surface cracks and bubbles create an additional number of air-solid interfaces so that x is greater than 2 and this fact would have to be taken into account in calculating C.

These difficulties were overcome by evaluating C from experimental data. Records for 90 disks, processed from the same semolina under identical conditions, and varying in thickness from 0.201 to 0.226 cm, gave a constant value of K (3.99) when $C = 3.03$. Using this figure the equation becomes:

$$-K = \frac{1}{t} \log_e 3.03 \times \frac{.194}{500} V_t$$

or

$$-K = \frac{1}{t} \log_e .00118 V_t. \tag{6}$$

The value obtained for C was checked with a series of 120 disks made from another semolina with different optical properties, namely, a mean absorption coefficient of 2.42. The disks were classified with

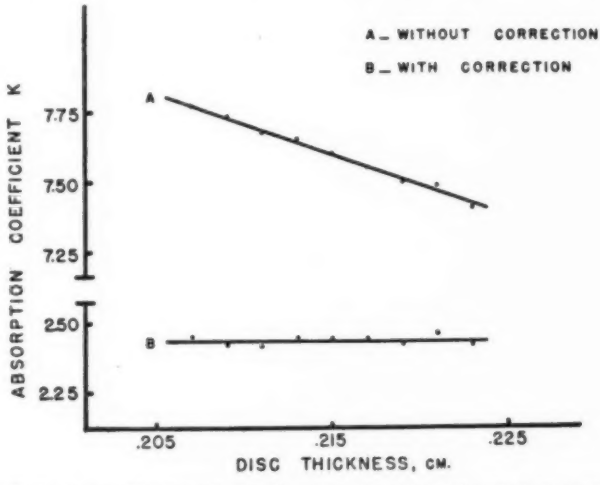

Fig. 4. The effects of correcting for reflection losses on the calculated optical absorption coefficient.

respect to thickness into nine groups, each of which had a range of 0.002 cm. The mean thickness for each group was then plotted against the mean absorption coefficient. Line A in Figure 4 shows the relation between the absorption coefficient and disk thickness when only the thickness correction is applied, *i.e.*, neglecting the factor C. Under these circumstances, the absorption coefficient appears to decrease with increasing thickness, even though a correction has been applied for differences in thickness. This must occur for the following reasons.

The loss of light by reflection at the upper surface is obviously the same for both thin and thick disks. A constant proportion of the light reaching the lower surface is also lost by reflection, but the amount of light reaching this surface is greater for thin than for thick disks. In consequence the absolute loss of light by reflection is greater for thin than for thick disks, and the absorption coefficient, or opacity, of a thin disk therefore appears to be higher than that of a thick disk.

When the reflection correction is applied (assuming that $C = 3.03$) the line B in Figure 4 is obtained. It shows that when the correction is made the absorption coefficient is independent of disk thickness as it should be with a series of disks made from the same semolina under identical conditions. Moreover, since the line is level, it is apparent that the value of C, derived from disks made from the first semolina, applies equally well to disks made from the second semolina, which has quite different properties. Thus, while on theoretical grounds it might be assumed that C would have a different value for different semolinas, either because of differences in indices of refraction or in number of air-solid interfaces, in practice it appears that a constant value for C can be used. This hypothesis has been checked with dozens of pairs of duplicate disks made from a variety of different semolinas. The use of a constant value of C to correct for reflection losses reduces the differences between the absorption coefficients for duplicate disks differing in thickness.

Accordingly, equation 6 is now being used in this laboratory for the calculation of absorption coefficients for all micro disks of alimentary paste. The calculation of K can be simplified by drawing a graph of $\log_e .00118 V_t$ for a wide range of voltages. The value obtained from this graph for the corrected voltage generated in the cell by the light transmitted by the test disks, when divided by the thickness of the disk, gives the absorption coefficient K of the material in the body of the disk.

Total Color Scores

When macaroni samples or micro disks are matched on a color comparator against the Wallace and Tiernan disks described by Baker (1939), four figures are obtained representing the percentages of black, white, yellow, and red used in matching the sample. No ordinary individual can create in his mind an image of the color of the disk by looking at these four figures, nor is it easy to determine by inspection of the data the order in which a series would be placed by visual comparison. Accordingly, it is frequently useful to summarize the data by combining the figures for black, white, yellow, and red into a total color score. This arbitrary procedure does not represent a fundamental approach

to the problem of assessing color characteristics, but can be defended to the extent that it yields figures correlated with visual placing. It has proved particularly useful for summarizing color data for plant breeders, grain inspectors, and other persons less conversant with the qualities of macaroni than the cereal chemists who have specialized on this product.

A formula for calculating a color score for macaroni was published by Binnington, Johannson, and Geddes (1939), but this proved to be unsatisfactory for the wide range of colors obtained in a study of the effects of processing factors on the quality of micro disks (to be described in a second paper), since it was found that white disks had a better score than translucent yellow ones. It was therefore necessary to design a formula which would hold over a wider range of color combinations.

The new formula is based upon the degree to which a disk fails to match an arbitrarily selected combination of colors, namely red 5%, yellow 40%, white 10%, and black 45%. The score is reduced by the square root of the sum of the weighted squares of the departures from the standard percentage of any color; thus negative and positive departures from the standard are given equal weight. It should be noted, however, that the choice of the percentages is such that the disks will normally require more than 5% of red and 10% of white, and less than 40% of yellow and 45% of black. The relative importance of departure with respect to each color is adjusted by multiplying the square of the departure by a constant. For example, it is considered about four times more detrimental to be 1% high in red than to be 1% high in white. The constant for red is thus 4, whereas that for white is 1. The formula tentatively adopted is as follows:

$$\text{Color score} = 100 - \sqrt{4(5-\text{R})^2 + 2(40-\text{Y})^2 + (10-\text{W})^2 + (45-\text{B})^2}.$$

This formula gives scores which are in good agreement with visual placing. Tests made with 13 samples of macaroni gave a correlation of 0.94 between the scores given by the new formula and by that of Binnington *et al.* The correlation between the new color score and the absorption coefficient is illustrated in Figure 5. The data represent series of disks processed by different methods from a single semolina and all are of the same pigment content. A more comprehensive study of this relationship will appear in a future paper.

It should be emphasized that the equation for the color score is developed in an arbitrary manner by trial and error and comparison of the results obtained with visual placing. The formula of Binnington *et al*, which involves a calculation of saturation, hue, and brilliance, and

a subsequent combination of these to give a total score, appears to represent a more fundamental approach to the problem of assessing color data. Actually it is no more fundamental or less arbitrary than the new formula. Both are developed by the same method; no theoretical support can be offered for the values given to the constants introduced into either equation, nor can they be supported by objective quanti-

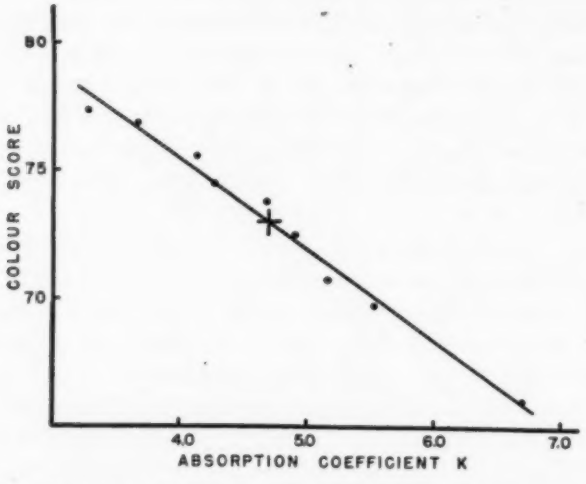

Fig. 5. The relationship of the new total color score to the optical absorption coefficient.

tative data (Binnington *et al*). They can be checked only against visual placing and this depends on subjective judgment. The only safeguard which can be used is that of comparing one's judgment with that of other experienced persons and this practice is followed periodically in the laboratory.

Relation Between Absorption Coefficients and Colors

When absorption coefficients and colors, for a series of disks made from the same semolina, were compared by plotting the former against percentages of black, white, red, and yellow, the graph shown in Figure 6 was obtained. This graph does not appear to support the hypothesis, mentioned earlier, that more opaque disks look less yellow because one cannot see as far into them. The curves show that the percentage of yellow increases instead of decreasing with increasing absorption coefficient. Moreover, the percentage of red shows only a very slight decrease with increasing opacity.

Further consideration led to a new method of interpreting results obtained with Wallace and Tiernan disks. Black does not reflect light. Accordingly, when a paste disk is matched by a certain combination of

the black, white, red, and yellow disks, the amount of light reflected by the paste is balanced by the amount of light reflected by the white, red, and yellow segments only. The adjustment of the black segment serves only to reduce or increase the total amount of light reflected, and

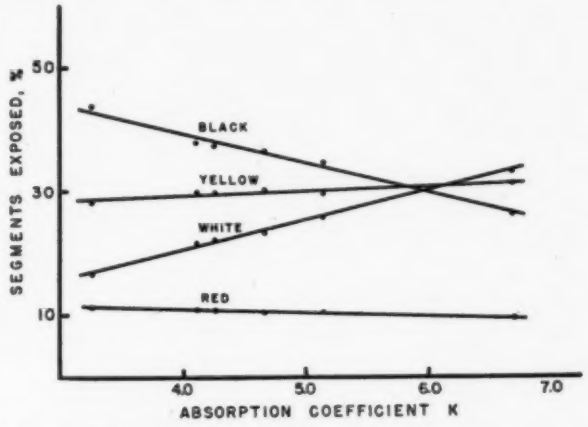

Fig. 6. The relationship of the optical absorption coefficient to the percentages of the matching segments exposed.

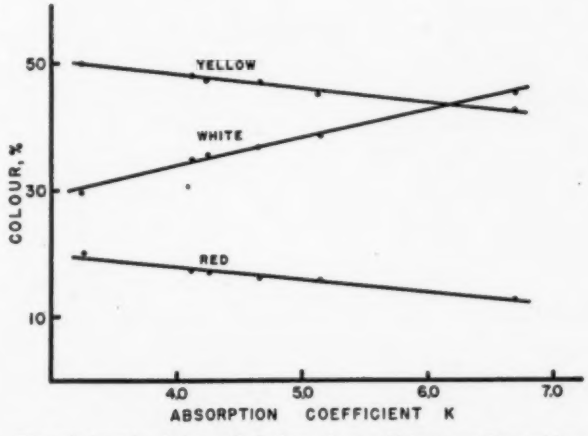

Fig. 7. The relationship of the optical absorption coefficient to yellow, white, and red expressed as percentages of the nonblack component.

percentage of black actually measures the inability of the paste disk to reflect the light.

It thus appears that a better understanding of the color of the paste can be obtained by considering the three colors as percentages of the nonblack component. When the data shown in Figure 6 are treated in this manner, the graph in Figure 7 is obtained. Both yellow and red now decrease with increasing opacity in accordance with the hypothesis

outlined above. It should be added that this decrease in yellowness with increasing opacity can be readily observed when the disks are compared visually. An average set of experimental data illustrating this point are shown in Table I. It will be observed that when the old method of recording color percentages is used the white disks are reported as containing a higher percentage of yellow than the yellow disk. The discrepancy disappears when the new method of recording

TABLE I

COMPARISON OF OLD AND NEW METHODS OF RECORDING COLOR DATA

Appearance of disk	Color as percent of all components				Color as percent of nonblack components		
	Yellow	Red	White	Black	Yellow	Red	White
	%	%	%	%	%	%	%
Whitish	31.4	9.1	33.1	26.4	42.6	12.4	45.0
Pale yellow	29.9	10.7	21.7	37.7	48.0	17.2	34.8
Yellow	28.2	11.3	16.8	43.7	50.1	20.0	29.9

color as percentages of the nonblack component is used. This fact alone provides strong support for the new method of interpreting color data.

The data shown in Figures 6 and 7, and in Table I, were obtained from disks processed from single samples of semolina. It therefore remains to be seen whether this additional method of interpreting color percentages will prove useful in studying the relative quality characteristics of different semolinas. The method is described in this paper merely because it serves to elucidate the relation which exists between the opacity and color characteristics of alimentary pastes.

Summary

The method of Fifield, Smith, and Hayes (1937) for preparing micro disks of alimentary paste has been modified in certain ways so as to increase the reproducibility of the test. The chief modifications involve the use of (1) a thermostatically controlled, motor-driven mixer, (2) motor-driven sheeting rolls, and (3) wax-coated celluloid instead of cellophane for covering the disks during pressing. A photometer is described with which a precise and objective measurement of the optical absorption coefficient (degree of opacity) of the disks can be made. The calculation of the coefficient involves certain difficulties and the derivation of the required formula is therefore described in detail. Determination of the absorption coefficient provides a new and useful method of assessing the quality characteristics of the disks. A formula for computing total color scores from data obtained by comparing paste

with Wallace and Tiernan disks is recorded and discussed. Study of the relation between the absorption coefficient and Wallace and Tiernan color data suggests a new method of interpreting the latter which appears to lead to a better understanding of the color characteristics of the paste.

Acknowledgment

The authors are indebted to Mr. V. G. Martin who constructed the photometer and mixer and had a large part in designing this equipment.

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MICRO MILLING AND BAKING OF SMALL SAMPLES OF WHEAT [1]

MAX E. MCCLUGGAGE [2]

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Estimating the baking characteristics of small lots of wheat is important in the development of new varieties by plant breeders. Consequently, there has been considerable demand for reliable methods of testing the smallest possible samples.

Geddes and Aitken (1935) developed a modified Allis experimental mill which was designed to mill a 100-g instead of the usual 2,000-g sample. They milled a series of wheats with it and also with the regular Allis mill and baked the resulting flours into bread. A 25-g dough and scaled-down equipment were used for the micro-milled flours, while the usual methods were used for the regularly milled flours. They found no evidence that the two milling methods produced flours of different baking characteristics. The principal objection to their methods appears to be the expense of constructing the mill.

[1] The studies reported herein are a part of the cooperative work carried on between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Agricultural Experiment Stations of the Great Plains Region. Published as Contribution No. 85 of the Department of Milling Industry, Kansas Agricultural Experiment Station.

[2] Formerly Agent, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Hard Winter Wheat Quality Laboratory, Kansas Agricultural Experiment Station, Manhattan, Kansas.

Van Scoyk (1939) reported that a micro method, using a 25-g dough and special equipment, gave results that compared favorably with those obtained by the usual methods.

Harris and Sanderson (1939) made a study similar to that reported by Geddes and Aitken and concluded that there were no significant differences in protein content, flour yield, and loaf volume that could be attributed to the two methods of milling.

Clark [3] demonstrated that he could obtain a meal, by grinding wheat on a laboratory grinder and sifting the ground material through a 10XX flour cloth, that produced dough-mixer curves resembling those obtained from a commercially milled flour. The material passing through the cloth was termed "wheat meal" and the curves were referred to as "wheat meal dough-mixer curves."

Swanson and Johnson (1941) developed a technique similar to that used by Clark for milling on a Hobart laboratory grinder. They found a high correlation between the characteristics of the curves produced from the wheat meal thus milled and those from regularly milled flour of the same wheat.

Any technique of micro milling and baking, if it is to be widely useful for plant breeders, must make use of inexpensive and easily obtainable equipment. A laboratory grinder to mill the wheat and the micro-baking techniques previously referred to meet these requirements. The purpose of this paper is to report the results of a study in which this combination was used.

Plan of the Work

The studies reported herein involve two phases of the same problem: (1) a preliminary study of the reproducibility of results from day to day with micro-baking techniques using two types of pans, and (2) a comparison of baking results obtained with the micro-milling and micro-baking techniques with those obtained by the regular procedures used in this laboratory.

In the preliminary study eight samples of wheat (chosen to cover a wide range of baking characteristics) were milled on three different days by the technique described herein. Each of the 24 flours was then baked as a single loaf on each of 3 days in a high and in a low pan. Forty-eight loaves were baked each day and a total of 144 loaves for the study.

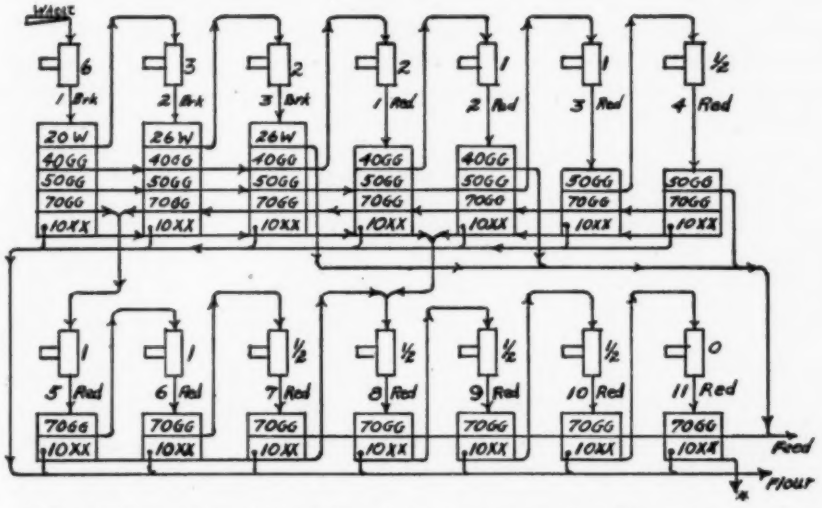
For the comparison of the micro and regular techniques a series of 25 samples was selected which had widely different baking characteristics. They were milled and baked by the micro techniques and the

[3] Personal communication from Mr. Rowland J. Clark, Director of Research, The Shellabarger Mill and Elevator Company, Salina, Kansas, dated August 15, 1939.

results were compared with those that had been obtained several months earlier by the regular procedure.

Techniques Used

Regular milling procedure: The samples were cleaned and scoured in the usual way before milling and tempered according to their requirements as described by McCluggage (1939). A 4,000-g sample of each wheat was milled on a Buhler mill to a straight grade flour as nearly comparable as possible with that which presumably would be secured under commercial conditions. The mill was set for each individual sample. The atmospheric conditions in the mill room were automatically controlled as nearly as possible to 70°F and 50% relative humidity.



* The mill is set with the grinding index on ¼ when burrs are running slightly together. The figure shown for each grinding is the approximate index setting. The overs of the 10 XX from the 11th reduction are re-ground until about 70% of the total products have been recovered as flour.

Fig. 1. Flow sheet used in milling samples on the Hobart laboratory grinder.

Regular baking procedure: The bakings were made with unbleached flour which had been stored at 70°F for three weeks after milling and then placed in cold storage at 40°F until baked. The following ingredients were used: 200 g flour, water as needed, 12 g sugar, 3 g salt, 4 g yeast, 6 g shortening, 8 g dry skim-milk solids, 0.5 g malt (120°L), and potassium bromate as needed.

The doughs were mixed to optimum development for each sample with a Swanson-Working mixer, and since the pans would accommodate only 100 g of dough, each was divided into equal parts and fermented at 86°F for 105 minutes to the first punch, 50 minutes to the

second punch, and 25 minutes to the pan. They were proofed at 86°F for 55 minutes and baked for 25 minutes at 420°F. The doughs were mechanically punched and molded. Duplicate bakes were made on separate days or a total of four loaves for each sample were baked.

Micro milling technique: The micro millings were made with a Hobart laboratory grinder, this being substituted for the regular experimental rolls. A flow similar to that used with the Allis mill was employed and is described in Figure 1. The sifting was done on a Roto-matic sifter. Swanson and Johnson (1941) used a limited number of grindings and obtained very low flour yields, but in the present study a sufficient number of breaks and reductions were used so that normal yields of approximately 70% were obtained.

TABLE I

EFFECT OF MILLING AND TYPE OF PAN ON MICRO-LOAF VOLUMES

Variety	Milling days	Loaf volumes (cc)							
		Baked in high pan				Baked in low pan			
		1st day	2nd day	3rd day	Av	1st day	2nd day	3rd day	Av
Kharkof	1st	190	200	190	193	205	203	195	201
	2nd	195	180	188	188	210	195	205	203
	3rd	190	195	190	192	205	195	180	193
Cheyenne	1st	180	210	170	187	210	215	180	202
	2nd	180	195	193	189	196	207	205	203
	3rd	190	190	195	192	205	200	195	200
Pawnee	1st	180	190	180	183	210	190	205	202
	2nd	190	193	175	186	195	187	195	192
	3rd	185	185	175	182	185	195	190	190
Tenmarq	1st	165	175	165	168	175	182	180	179
	2nd	150	158	165	158	180	165	175	173
	3rd	160	165	157	161	165	170	170	168
Kharkof	1st	140	147	150	146	150	155	147	151
	2nd	150	160	157	156	150	160	147	152
	3rd	140	145	140	142	150	152	150	151
Pawnee	1st	145	145	135	142	150	150	145	148
	2nd	140	140	140	140	150	150	150	150
	3rd	140	140	135	138	150	150	147	149
Comanche	1st	140	145	135	140	150	153	147	150
	2nd	150	142	145	146	150	145	145	147
	3rd	140	143	130	138	150	145	150	148
Nebred	1st	210	212	210	211	218	235	235	229
	2nd	190	210	205	202	235	235	210	227
	3rd	195	202	205	201	205	223	202	210

Each 300-g sample of wheat was tempered to 16% moisture and allowed to stand overnight. The grinder was adjusted for each sample and for each step of the flow sheet. The final reductions and siftings were continued until approximately 70% of the total products were obtained as flour. The grinder settings shown on the flow sheet are, of course, only approximate.

In using the laboratory grinder as a mill it was found necessary to avoid overloading the grinder to prevent overheating of the samples. The grinder was thoroughly cleaned between samples to avoid contamination of one sample by another.

Micro baking techniques: Twenty-five grams of flour was mixed and baked, with the same proportions in the formula as were used for the regular baking procedure. The doughs were mixed in a National non-recording micro mixer, fermented in 250-ml glass beakers covered with watch glasses, and were punched and molded by hand. The pans were the official A.A.C.C. style, only reduced to one-fourth the usual volume.[4] The loaf volumes were measured in a National volume meter by inserting a 400-cc dummy with the loaf.

TABLE II

ANALYSIS OF VARIANCE OF DATA SHOWING EFFECT OF MILLING, BAKING AND TYPE OF PAN UPON MICRO-LOAF VOLUMES

Cause of variance	Degrees freedom	Sum of squares	Mean squares	*F*
Sample (S)	7	90,365	12,909.4	251.0*
Millings (M)	2	608	304.0	5.9*
Bakings (B)	2	625	312.5	6.1*
Pans (P)	1	3,640	3,640.0	70.9*
1st order error	131	6,733	51.4	—
S × M	14	799	57.1	1.2†
S × B	14	849	60.6	1.3†
S × P	7	537	76.7	1.6†
M × B	4	223	55.8	1.2†
M × P	2	68	34.0	0.7†
B × P	2	174	87.0	1.9†
2nd order error	88	4,083	46.4	—
Total	143	101,971	—	—

* Highly significant value.
† Nonsignificant value.

Experimental Results

The results of the preliminary study are presented in Table I. Since the baking characteristics other than loaf volumes were not differentiated, loaf volume data only are given. Analysis of variance was applied to the data and the results are tabulated in Table II. It

[4] Acknowledgment is made of the loan of the pans used in this study by Mr. R. M. Sandstedt, Nebraska Agricultural Experiment Station, Lincoln, Nebraska.

will be seen that there were significant differences between millings on different days and also between bakings made on different days. The greatest differences, however, were related to the type of pan. It seemed advisable, therefore, to apply analysis of variance separately to the data for each type of baking pan. This was done and the resulting data are given in Table III.

TABLE III

ANALYSIS OF VARIANCE OF DATA SHOWING EFFECT OF MILLING AND BAKING UPON MICRO-LOAF VOLUMES WHEN BAKED IN TWO TYPES OF PANS

Cause of variance	*DF*	Baked in high pan		Baked in low pan	
		Mean square	*F*	Mean square	*F*
Sample (S)	7	5,740	130.0*	7,302	140.4*
Bakings (B)	2	252	5.7*	121	2.3†
Millings (M)	2	68	1.5†	.234	4.5*
1st order error	60	44		52	
S × B	14	44	1.2†	63	1.2†
S × M	14	55	1.5†	51	1.0†
M × B	4	57	1.5†	56	1.1†
2nd order error	28	37	—	52	
Total	71	—	—	—	—
Between samples	7	5,740	110.4*	7,245	114.1*
Within samples	64	52	—	64	
Total	71	—	—	—	—

* Highly significant value.
† Nonsignificant value.

The loaf volumes obtained with the low pans were significantly larger than those obtained with the high pans, but the variance was also greater, which suggests that both types were equally efficient in differentiating between varieties. None of the interactions was significant. Hence it would seem that either pan might be used with satisfactory results. It may be noted, however, that when considered separately the low pans indicated significant differences between day-to-day millings, whereas the high pans did not, and conversely that the high pans indicated significant differences between day-to-day bakings, whereas the low pans did not. No logical explanation for this differential reaction seems apparent. On the assumption that there was a real though unknown reason for the difference, the high pans only were used in subsequent work, since it is possible to replicate bakings with samples of wheat so small that replicate millings cannot be made.

Comparison of Micro and Regular Techniques

Table IV gives the pertinent analytical data for the wheat and flour and the baking results necessary for a comparison of the micro and the regular techniques. Each loaf volume reported is an average of two bakes.

The ash of the micro-milled flours was much higher than is usually expected from flours milled to a 70% yield. The protein content was

TABLE IV

CHEMICAL [1] AND BAKING DATA FOR THE WHEATS AND FLOURS MILLED BY THE MICRO AND REGULAR TECHNIQUES

Variety	Source of samples	Wheat			Buhler flour				Micro-milled flour			
		Moisture	Ash	Protein	Moisture	Ash	Protein	Loaf volume	Moisture	Ash	Protein	Loaf volume
		%	%	%	%	%	%	cc	%	%	%	cc
Kharkof	Central Great Plains	10.9	1.70	16.4	14.4	.43	15.6	1149	13.4	.83	17.0	194
Blackhull		10.9	1.68	16.2	14.3	.42	15.6	1079	12.8	.70	16.5	198
Tenmarq		10.9	1.68	15.7	14.2	.45	14.8	1003	13.8	.88	16.0	188
Oro		11.1	1.75	16.2	14.9	.45	15.3	1142	14.2	.83	16.7	205
Cheyenne		11.0	1.67	15.8	14.7	.43	15.0	1003	13.5	.81	16.2	186
Pawnee		11.1	1.53	15.9	14.5	.42	14.3	1036	13.2	.81	16.5	193
Comanche		11.2	1.61	16.3	14.3	.39	15.3	1047	13.1	.77	16.5	193
Chiefkan		10.7	1.66	15.8	14.0	.41	14.8	823	13.0	.82	16.0	164
Kharkof	Southern Great Plains	11.4	1.64	13.9	14.6	.45	12.6	859	13.6	.83	13.6	164
Blackhull		11.4	1.73	13.6	14.4	.39	12.7	831	14.2	.68	13.6	164
Tenmarq		11.3	1.57	14.0	14.4	.40	12.3	878	12.6	.83	13.7	170
Chiefkan		11.5	1.64	13.7	14.3	.43	12.8	720	12.9	.85	13.9	150
Kharkof	Northern Great Plains	9.6	1.46	13.0	13.9	.45	12.5	828	12.9	.80	13.3	155
Nebred		9.4	1.44	13.1	14.1	.42	12.7	846	12.6	.68	13.2	175
Blackhull	Denton, Texas	—	—	—	14.5	.38	9.8	692	12.8	.94	10.7	145
Tenmarq		—	—	—	14.9	.47	9.7	688	13.3	.83	10.0	147
Pawnee		—	—	—	14.9	.45	9.8	680	13.1	.82	10.3	145
Comanche		—	—	—	14.4	.49	10.1	725	13.4	.80	10.4	148
Commercial samples												
No. 3 D.H.W.	Kansas City	8.7	1.78	14.8	15.0	.44	13.8	825	12.3	.93	15.1	165
No. 2 D.H.W.	Fort Worth	8.7	1.72	15.3	14.6	.45	14.5	973	12.4	.86	15.6	180
No. 1 D.H.W.	Wichita	9.4	1.60	12.5	14.7	.42	11.6	780	12.5	.78	12.7	156
No. 1 D.H.W.	Enid	9.1	1.61	13.9	14.4	.41	13.1	885	12.7	.86	14.4	183
No. 2 H.W.	Chicago	9.0	1.69	10.0	15.1	.37	9.6	710	12.8	.80	10.7	154
No. 2 H.W.	Minneapolis	9.5	1.72	10.7	14.9	.43	9.4	686	13.0	.66	9.8	144
No. 2 H.W.	Portland	9.2	1.45	9.5	14.0	.41	8.4	653	12.3	.82	9.5	130

[1] Ash and protein values are expressed on a 13½% moisture basis.

generally higher than that in the Buhler-milled flours and in most cases as high as that found in the wheat from which the flour was milled, or higher.

In spite of these differences in chemical composition the loaf volumes from the two flours agreed remarkably well as is shown by the correlation coefficients in Table V. All coefficients were so high that there can be no doubt as to their significance. The agreement is shown graphically in Figure 2 in which the micro loaf volumes are plotted against the regular 100-g loaf volumes.

TABLE V

CORRELATION COEFFICIENTS FOR CHEMICAL AND MILLING DATA OF WHEATS MILLED AND BAKED BY THE MICRO TECHNIQUE (HOBART LABORATORY GRINDER) AND THE REGULAR LABORATORY PROCEDURE (BUHLER MILL)

Factors correlated	Coefficient of correlation r	Regression coefficient b
Wheat protein and Hobart flour protein	+.99	1.07
Wheat protein and Buhler flour loaf volume	+.90	—
Wheat protein and Hobart flour loaf volume	+.89	—
Hobart flour protein and Hobart flour loaf volume	+.90	—
Buhler flour loaf volume and Hobart flour loaf volume	+.97	7.24

All the points in Figure 2 except six fell so close to the regression line as to be within the experimental error (25 cc) of the 100-g loaf volumes. There is some indication that these six points were the results of random errors, since they were not grouped in any particular part of the loaf-

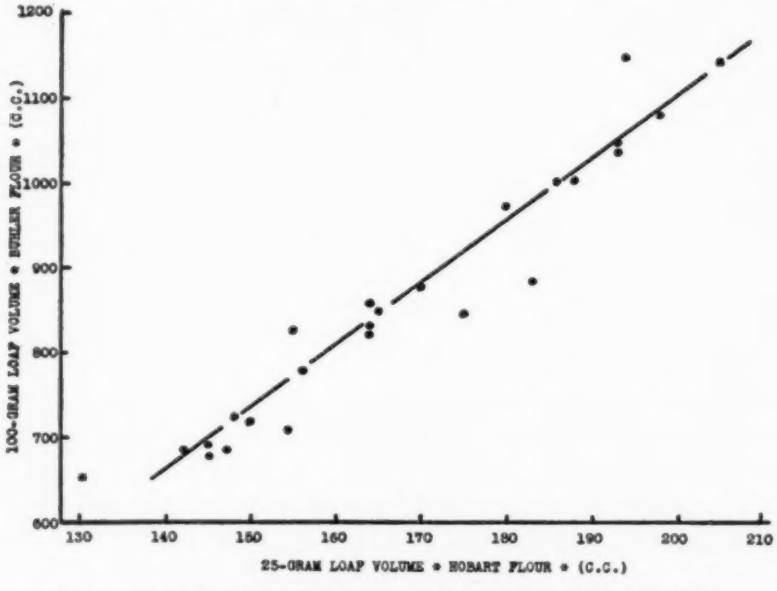


Fig. 2. Loaf volumes by the Hobart-micro method and the Buhler 100 g method.

volume range and half the points were above and half below the regression line.

The regression equation (100-g loaf volume = 7.24 micro-loaf volume − 347) is represented by the solid line. This equation was used to calculate the expected 100-g loaf volumes. The standard error of estimate was found to be 39 cc, as compared to the 25-cc error of replication for the 100-g loaf volumes. If a check sample of known

characteristics is included in each series of samples of unknown quality, their relative merits can be determined by direct comparison and the estimated 100-g loaf volume need not be calculated.

Summary

The data presented show that a fairly accurate picture of the baking characteristics of wheat samples may be obtained by the micro-milling and micro-baking techniques described herein.

The techniques described in this paper are not proposed as short cuts. The time required for testing samples by these methods is approximately the same as for the regular laboratory procedures.

One advantage of the micro methods is that only about one pound of wheat is required, as compared with the usual five-pound sample. Another advantage is that the regular laboratory wheat grinder is used for the milling, thus eliminating the necessity of owning a micro experimental mill, which is expensive. It should be possible to develop small-size fermentation cabinets and baking ovens so that the entire equipment for milling and baking would require very small space.

This method is of importance to any plant breeder who has a few samples to test each year and desires baking information in the early stages of development of new varieties of wheat. This technique offers each plant breeder an opportunity to make baking determinations without investing large amounts of space, equipment, and material.

Acknowledgment

The author wishes to acknowledge the assistance of Mr. Arthur Hibbs who did the micro milling, and Mr. Karl F. Finney whose data obtained by the regular baking procedure were used for comparison.

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YEAST VARIABILITY IN WHEAT VARIETY TEST BAKING[1]

K. F. FINNEY and M. A. BARMORE[2]

(Read at the Annual Meeting, May 1942)

From the time the Hard Winter Wheat Quality Laboratory first began to use the rich, highly bromated formula giving large loaf volumes it was noted that occasionally the volumes for a bake of various samples would be from 0 to 200 cc below those for the same samples baked on another day. The amount of volume decrease seemed to depend, for the most part, upon the protein quality and content of the varieties being tested. Such results were at first thought to be due to lack of proper temperature control or to hand manipulation of the dough. After the installation of accurately controlled refrigeration and heating units as well as mechanical dough-handling equipment, however, such discrepancies continued to occur. It was later noted that when such irregular baking results occurred, each replicate had been baked with a different lot of yeast. Since then all yeast supplies have been tested for baking uniformity, using the same flour. By discarding all lots of yeast that tested below normal, the variability between replicate bakes on different days has been materially reduced. This paper is a report of the variations encountered and the effects of such in variety test baking.

The commercial baker deals with flours which average lower in protein and show less variation in protein quantity and quality than those encountered in the experimental baking of varieties. In the latter type of work one may encounter flours with a range of 10% in protein, and varying in quality from those that are very strong to those too weak for normal and satisfactory commercial use.

Varieties differing in protein quality and in physical and chemical properties respond differently to various baking ingredients. This variation in response becomes greater as the volume level rises, the volume level being a function of quality and quantity of protein. It is therefore natural to expect that as the volume level becomes greater there will be larger differences between duplicate bakes when using yeasts of varying strength. Yeast variations which would pass unnoticed by the commercial baker may then become of significant importance in studies dealing with wheat variety flours, especially in the higher volume levels.

[1] The studies reported herein are a part of the cooperative work carried on between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. D. A., and the Agricultural Experiment Stations of the Great Plains Region. Published as contribution No. 82 of the Department of Milling Industry, Kansas Agricultural Experiment Station.

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Experimental bakers have pointed out variations in yeast activity. Studies such as those of Harrel (1926), Cook and Malloch (1930), Weaver, Talbot, and Coleman (1933), Bailey, Bartram, and Rowe (1940), and Iwanowski and Brezezinski (1934, cited by Bailey *et al*), clearly demonstrate that the activity of yeast, like other biological materials, is affected by its environment and that it is particularly influenced by extremes of temperature.

Cook and Malloch (1930), in addition, found that the gas production of different samples of the same brand of yeast varied considerably. The studies of Weaver *et al* (1933) also included two brands of yeast; as a result of their work they recommended that more uniformity between different brands was advisable for experimental test baking.

Testing of yeast was carried out with the usual straight-dough method and what was considered an approximately optimum baking formula containing the following ingredients per loaf: 100 g flour, water as needed, 6 g sugar, 1.5 g salt, 3 g shortening, 2 g yeast, 4 g milk solids, 0.25 g 120°L malt syrup, and 3 mg $KBrO_3$. In these studies the various lots of yeast were stored at a temperature of 35°F during the intervals between baking tests.

The A. A. C. C. baking test procedure was used in conjunction with optimum mixing time. The advantages of the rich, highly bromated formula have been pointed out by the authors (1939, 1941). The procedure has been to test two lots of yeast at a time, *i.e.*, one was being tested for the first time while the other, under test for the second time, had reacted normally in previous tests and was therefore being used in the regular baking. Three replicates of each lot were baked on the same day using a uniform lot of flour containing 13.5% protein. The supply of flour was kept at 35° to 40°F. This method of testing yeast, although requiring additional work, can be carried out simultaneously with the routine baking schedule which is in no way interrupted as might be the case if tests of a different nature were involved.

The loaf volumes secured from the same uniform lot of flour but with different lots of yeast ranged from 855 to 980 cc. The variation was greatest in the summer and early fall months, suggesting lack of uniformity in storage conditions after manufacture as a probable cause. About 20 of the different lots of yeast secured during a period of nearly four months produced loaves averaging 963 cc with all averages for each lot varying no more than ± 20 cc from this value. Only those yeasts testing 960 ± 20 cc were considered "normal" and therefore used in regular baking work.

Figure 1 shows the time in days before the lot of yeast designated as A showed deterioration as measured by the loaf volumes of the uniform lot of flour referred to above. The results indicate extremely good

stability and keeping qualities for at least 18 days. By the twenty-seventh day, however, this yeast showed definite deterioration, giving results similar to those shipments which tested below normal upon arrival at the laboratory.

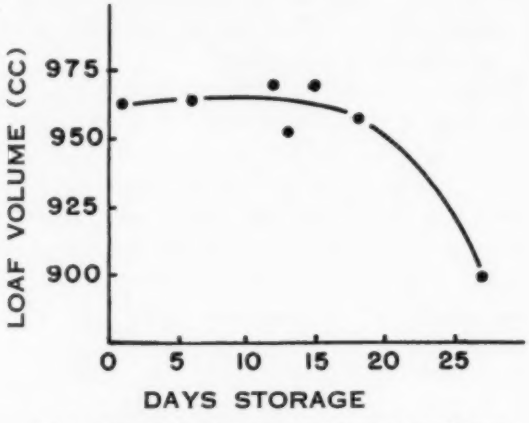

Fig. 1. Time in days before yeast A showed deterioration.

Figure 2 shows the variation in loaf volumes produced by different lots of yeast (designated as A, B, C, D, and E) used in concentrations of 2.0, 2.5, 2.75, 3.0, and 3.5%. The variation from the subnormal of 855 cc to the normal of 963 cc, at a concentration of 2%, indicates the

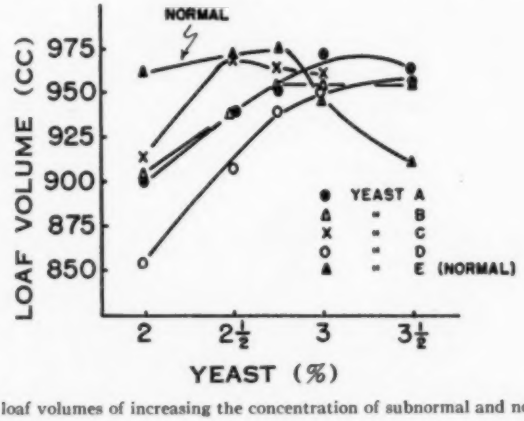

Fig. 2. Effect on loaf volumes of increasing the concentration of subnormal and normal lots of yeast.

variation in yeast strength encountered when using this concentration in the formula. Yeast A is from the same lot that produced the data shown in Figure 1, but after it had been kept for 27 days. Lots B, C, and D were fresh shipments of yeasts representing various degrees of subnormality. Two % of yeast E produced loaves that averaged

963 cc which is the average of the 20 lots mentioned above. Yeast E is therefore considered normal.

The curves of Figure 2 indicate that the yeasts which were lacking in ability to develop normal loaf volumes at 2% concentration, can be corrected for by increasing their concentration in the formula. It should also be noted that increasing the concentration of the normal yeast E above 2¾% resulted in a reduction of loaf volume. Such results are, for the most part, a function of the formula used. The conclusions from investigations in progress in this laboratory are that the action of yeast and bromate, as well as fermentation time, are complementary to a large extent in the securing of optimum baking results.

That subnormal yeast can be corrected for by increasing the concentration in the formula is further substantiated and probably better illustrated by the results shown in Figure 3 obtained with (part B) and without (part A) increasing the concentration of subnormal yeast. Part A of Figure 3 shows the results obtained when 10 different lots of

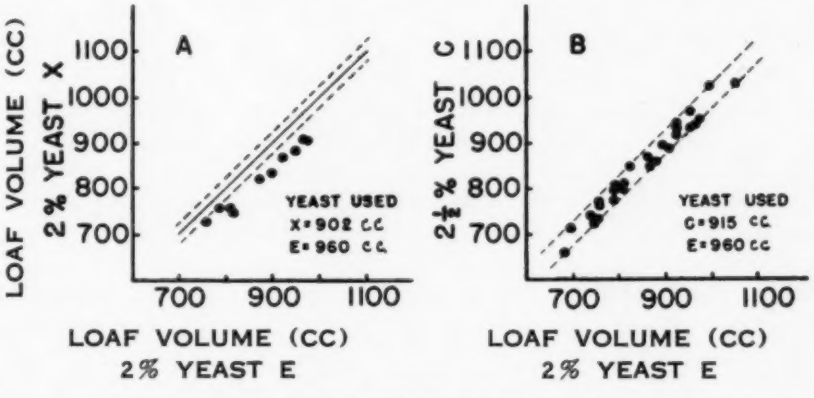

Fig. 3. Shows that by increasing the concentration of a sub-normal yeast, normal load volumes can be obtained.

flour representing a large range in loaf volume were baked with 2.0% of a normal yeast E and with 2.0% of a subnormal yeast X, testing 902 cc (about 60 cc low). The solid line locates where the points should fall when plotting the loaf volumes obtained with two normal lots of yeast. The allowable deviations from the solid line due to experimental error are defined by the broken lines. Actually, however, the regression coefficient for these points is 0.85 instead of 1.0, and the volumes are penalized from 30 cc at the lower volume level to 70 cc at the higher level as a result of using the subnormal yeast X. These data show that significant loaf-volume discrepancies between replicates resulted from using equal concentrations of yeasts of unequal strength.

The data including the regression coefficient of 0.85 also show that the inferiority of poor yeast is magnified in the higher volume levels.

The same ten samples of flour represented in part A of Figure 3, plus 20 additional ones, were baked with 2½% of a similar subnormal yeast C testing 908 cc (about 50 cc low) and with 2.0% of the normal yeast E testing 960 cc. Although yeasts X and C were not identical, each represented about the same degree of subnormality. The solid line in part B of Figure 3 indicates where the points should fall if 2½% of the subnormal yeast C produces loaf volumes identical to those obtained with 2.0% of the normal yeast E. The allowable deviations from the solid line due to experimental error are defined by the broken lines. The regression for these points is 0.998. These data show that completely normal loaf volumes were obtained, regardless of volume level, by increasing the concentration of the subnormal yeast C.

A third study was made to determine the effect of using subnormal yeast in the evaluation of varieties, especially at different protein levels. For this purpose the following samples were used: the varieties Tenmarq, Comanche, and Chiefkan, grown at several experiment stations in the Great Plains in 1939, and six station composites each of which was composed of equal parts of Turkey, Blackhull, Tenmarq, Nebred, and Chiefkan grown in Kansas. The formula used was the same rich, highly bromated formula used in yeast testing except that 4 mg of $KBrO_3$ was used for the composite protein series and 5 mg for the Tenmarq, Comanche, and Chiefkan series. Standard A. A. C. C. baking test procedures were used in conjunction with an optimum mixing time. Each sample was baked with a normal and a subnormal yeast. The subnormal yeast used produced an average loaf volume of 898 cc (65 cc low) with the uniform standard flour described earlier in this paper.

The results are shown in Figure 4, with loaf volumes along the ordinate and protein content on the abscissa. It is at once apparent that markedly different results were secured in most cases with the two lots of yeast. In general, the difference between the normal and subnormal yeasts increases with the protein content of the flour, undoubtedly because the loaf volume level increases markedly with protein content. The smaller volume differences for Chiefkan at any protein content would be expected because of its lower volume level.

These results suggest that 2% of the normal yeast resulted in more development of the protein than the same concentration of subnormal yeast and was more nearly optimum. Also, it is quite generally recognized that as the protein content increases within a variety, the fermentation and/or oxidation requirement increases, thus accounting for the greater loaf volume decreases in the high-protein or high-volume

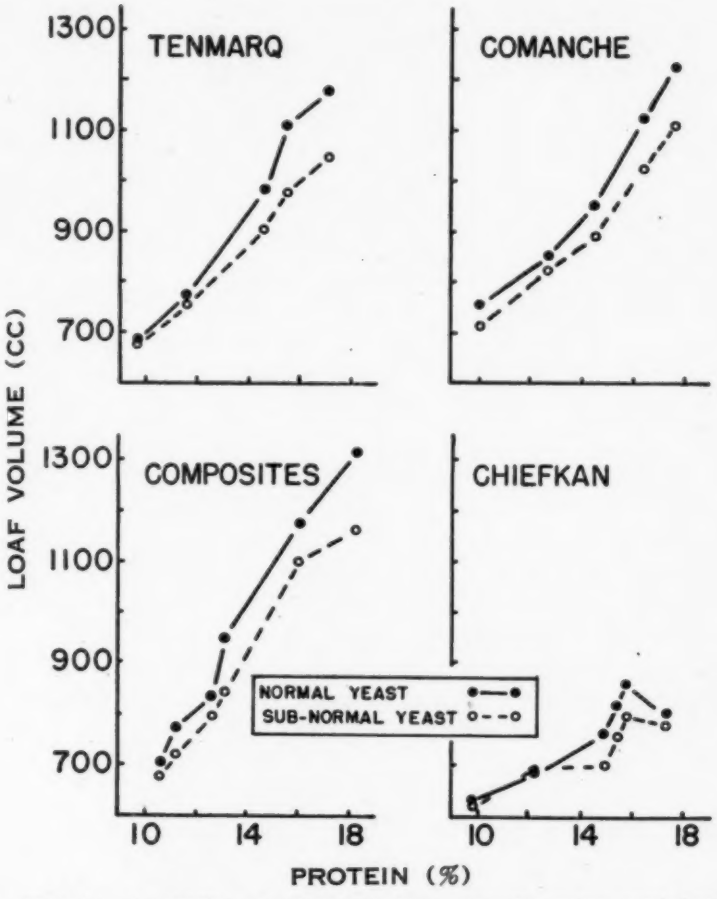

Fig. 4. Effect of yeast variability on loaf volume for flours representing various protein levels using the rich, highly bromated formula.

levels when using subnormal yeast. These results obtained with low-strength yeasts are similar to those frequently obtained with experimentally milled flours baked with formulas deficient in $KBrO_3$. The volumes obtained at the low protein levels indicate that the average variations in yeast would probably pass unnoticed by the commercial baker, since he generally works with flours containing 12% to 13% protein.

Serious error followed by erroneous conclusions can result from the occasional use of a subnormal yeast. For example, varieties grown in one environment and baked with a subnormal yeast would probably appear definitely inferior to the same varieties from another location when baked with normal yeast. Similar inconsistencies would be expected for varieties baked with a subnormal yeast one year and

with a normal yeast the next. Undoubtedly yeast variability has been an important factor contributing to inconsistent results in the past. The amount of error introduced by using nonuniform yeast supplies will depend, for the most part, upon the degree of variability existing in the yeast used as well as upon the flour and its protein content.

Summary

Experiments reported herein show that considerable variability in experimental baking resulted with nonuniform yeast. A uniform sample of flour stored at 35°F produced, over a period of about four months, loaf volumes varying from 855 to 980 cc depending on the lot of yeast used.

Flours representing a wide range in protein content for each of several hard red winter wheat varieties were baked with normal and subnormal yeast in a rich, highly bromated, milk-containing formula.

Loaf volume discrepancies resulting from the use of subnormal yeasts were greatest for the higher protein levels and for varieties that normally produce high loaf volume. The degree of variability existing in the yeast used was also an important factor influencing the volume discrepancies. The data show that distinctly misleading results may be secured through failure to consider variation in yeast.

Normal, fresh yeast was found to retain its baking properties unimpaired for at least 18 days when stored at 35°F, but by the twenty-seventh day showed definite deterioration.

The results show that adjustments can be made for subnormal yeasts by increasing their concentration in the formula in proportion to the degree of subnormality. Thus a uniform and apparently optimum amount of dough development was obtained. The procedure adopted in this laboratory is to test each lot of yeast by making replicate bakes with a uniform lot of flour kept for the purpose and discarding all lots of yeast that fail to meet predetermined standards.

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THE USE OF OXIDIZING AGENTS IN THE REMOVAL OF INTERFERING COMPOUNDS IN THE DETERMINATION OF NICOTINIC ACID

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The determination of nicotinic acid in cereal products is made difficult by the presence of interfering compounds encountered in the preparation of extracts of these products. Because of these compounds, conflicting results are reported for the nicotinic acid content of cereal products. Results from dog bioassay, chemical, and microbiological methods show no agreement as to the content of nicotinic acid in cereal products.

By the application of different chemical procedures for the determination of nicotinic acid in cereal products, Brown, Thomas, and Bina (1942) have shown that interfering substances are present in the extracts of cereal products. Accordingly, the chemical procedures must be designed to eliminate this interference in the determination of nicotinic acid, or otherwise abnormally high values will result. Two procedures were shown to be effective in eliminating these compounds: (1) extraction by ethyl acetate of the color complex that is formed in the König reaction between nicotinic acid, *p*-aminoacetophenone, and cyanogen bromide, and (2) the destruction by oxidation of the interfering substances with hydrogen peroxide. The oxidized extracts gave the same value, whether determined on the aqueous phase with aniline or on the ethyl acetate phase with *p*-aminoacetophenone.

Harris and Raymond (1939) were the first to show that ethyl acetate could be used to extract the color complex when *p*-aminoacetophenone was used as the aromatic amine in the König reaction. These authors, however, failed to apply this modification in their published work but they, as well as Kodicek (1940), recognized that cereal products contain chromogens that interfere with the determination of nicotinic acid. Arnold, Schreffler, and Lipsius (1941) used this modification in their published procedure for the determination of nicotinic acid. Bina, Thomas, and Brown (1941) recognized the value of this solvent in completely extracting the color complex of nicotinic acid and *p*-aminoacetophenone and in separating the complex from interfering chromogens.

As a result, they adopted it as a part of their procedure. The fact that only a portion of the total color developed in cereal extracts is extractable with ethyl acetate, while all of it is extracted from yeast

or pure nicotinic acid with this solvent, was convincing proof to these authors that compounds with chemical properties different from nicotinic acid were present and responsible for a part of the color measured as nicotinic acid by other procedures.

One of the characteristic chemical properties of pyridine compounds is their marked stability towards energetic chemical reagents. Only the side chains are attacked by oxidizing agents, with the formation of the corresponding acids. If a precursor exists, nicotinic acid will be produced, not destroyed, by oxidation, and whatever nicotinic acid is present will remain. In studying the nature of the interfering compounds encountered in the determination of nicotinic acid in cereal products, Brown, Thomas and Bina (1942), proceeding on this basis, and taking advantage of the well-known stability of nicotinic acid towards oxidizing reagents, used hydrogen peroxide to decolorize and remove the readily oxidizable compounds contained in the extracts of cereal products. These authors showed that ethyl acetate and hydrogen peroxide served the same purpose, but in different ways, in the determination of nicotinic acid in cereal products. Both procedures gave the same result for nicotinic acid, and both showed that the chemical properties of nicotinic acid were different from the chemical properties of the interfering chromogens from which it was separated.

In this paper we present the results of analyses made on cereal extracts before and after treatment with oxidizing reagents. The assays were carried out on aliquots of the same samples by both chemical and microbiological methods.[1] The chemical methods used were the procedures of Bina, Thomas, and Brown (1941) and a procedure where aniline is used instead of *p*-aminoacetophenone. The microbiological method was that of Snell and Wright (1941). The results obtained by the microbiological method show the presence of interfering substances that affect the values for nicotinic acid in a manner corresponding to similar effects in the chemical methods. Also some of these extracts before or after treatment with hydrogen peroxide gave results by the microbiological method that were of the same magnitude as those published by Thomas, Bina, and Brown (1942) in which ethyl acetate was employed to extract selectively the nicotinic acid color complex, formed with cyanogen bromide and *p*-aminoacetophenone, from other chromogens. Other extracts behave differently. Of the various aromatic amines which have been employed in the König reaction, *p*-aminoacetophenone is the only one yielding a color complex selectively extractable with ethyl acetate.

[1] The determinations by the microbiological procedure were made by Dr. John Rehm and Mr. M. L. McCormack of the Bacteriological and Pure Culture Laboratories of Anheuser-Busch, Inc. The authors wish to acknowledge their valuable assistance.

Experimental

Whole wheat flour: Material for these experiments was the whole wheat flour sample sent out for collaborative assay by Dr. John Andrews. Samples for analyses were prepared as follows: 20 g of the finely ground whole wheat flour was mixed with 100 ml of water and the mixture autoclaved for 30 minutes at 15 pounds pressure. The mixture was cooled to 50°–60°C, treated with 0.5 g of takadiastase for 30 minutes, and again autoclaved. The extract was separated from the insoluble material and the residue washed twice with water. The combined extracts and washings were hydrolyzed with hydrochloric acid in accordance with the regular procedure of Bina, Thomas, and Brown (1941). The neutralized extract was made up to a volume of 250 ml. Aliquots of this solution were used for analyses before and after oxidation treatment. These extracts contain considerable chromogen other than nicotinic acid.

The oxidation treatment was as follows: 10-cc portions of the original extract were acidified by the addition of 1 ml of concentrated hydrochloric acid and 5 ml of Superoxol (30% hydrogen peroxide solution) and heated in a water bath at 60°–70°C as long as oxygen was given off. The water-bath temperature was gradually brought to the boiling point. The solution was cooled to 60°–70°C, 5 ml more of Superoxol was added, and the treatment continued until the solution was evaporated. A strongly colored residue was obtained. This was dissolved in 20 ml of distilled water and neutralized. The solution

TABLE I

COMPARATIVE TREATMENTS WITH WHOLE WHEAT FLOUR

Method	Original extract	Peroxide treatment	H_2O_2 and Lloyd's reagent treatment
	μg/g	μg/g	μg/g
Bina, Thomas, and Brown	25.87	25.98	26.13
Snell and Wright	43.80	24.75	23.75
Aniline method	46.80	27.53	31.39

was again decolorized by treatment with 5 ml of hydrogen peroxide and evaporated to dryness on the steam bath. A colorless residue of salts was obtained giving a water-clear solution when dissolved in distilled water. This treatment usually removes all traces of hydrogen peroxide which affect the color determination by the chemical methods. Apparently traces of peroxides have no effect on the microbiological results. The solution at this stage may also be treated by Lloyd's reagent according to Dann and Handler (1941). The assay results on these solutions are shown in Table I.

These results show that the microbiological (Snell and Wright) method for the determination of nicotinic acid is not specific for this vitamin, and the data obtained on cereal products with this method do not correctly indicate a measure of the nicotinic acid content of whole wheat flour.

The original extract gave 38 μg/g when tested by Harris and Raymond's method (1939) using *p*-aminoacetophenone. The difference between this value and 25.87 μg/g in the Bina, Thomas, and Brown procedure is due to the ethyl acetate extraction.

A second series of experiments was made in which another extract of the same whole wheat flour was prepared as above and aliquots were used for analyses where the oxidation was made as follows: a 25-ml portion of the original extract was made alkaline by the addition of 1 ml of a 15% sodium hydroxide solution. Ten ml of Superoxol was added and the reaction allowed to proceed at room temperature. The reaction begins immediately upon the addition of the hydrogen peroxide and proceeds with a rise in temperature of the solution and the evolution of considerable gas. After approximately 15 minutes the vigorous reaction ceases and the solution is placed on a hot plate and boiled for several minutes. The solution was neutralized and analyses made as shown in Table II.

TABLE II

RESULTS OF ANALYSES FOR NICOTINIC ACID IN WHOLE WHEAT FLOUR

Method	Original extract	Oxidized extract
	μg/g	μg/g
Snell and Wright	40	48
Aniline method	51	35

These results are interesting in that the treatment increased the values for nicotinic acid by the microbiological procedure by 20% and reduced the values by the aniline method by 31.4%. The two methods did not measure the same material, because the stimulant responsible for the increase in the microbiological result is not a chromogen in the König reaction.

Wheat germ: A sample of commercial wheat germ was prepared for analysis by the same procedure as described for whole wheat flour. In addition to the peroxide oxidation, portions of the extract were also oxidized with potassium permanganate. The results obtained are shown in Table III.

The values obtained for nicotinic acid by the microbiological method (Snell and Wright) check very closely on the original and oxidized extracts for this wheat germ. These results, however, were appreciably lower than the values obtained by the chemical methods.

TABLE III

NICOTINIC ACID ANALYSES OF WHEAT GERM WITH COMPARATIVE TREATMENTS

Method	Original extract	Hydrogen peroxide	Potassium permanganate
	μg/g	μg/g	μg/g
Bina, Thomas, and Brown	56.0	53	—
Snell and Wright	35.6	38	—
Aniline method	84.5	49	56.2

This is true of the oxidized extracts where the interfering chromogens have been removed. The fact that approximately the same values were obtained on the original and oxidized extracts by the microbiological method, show that oxidation does not destroy any nicotinic acid present in the extract.

Wheat bran: Wheat bran free of screenings was used for these experiments. This material was exceptionally clean and was as free of germ and endosperm as commercial milling permits. Samples were prepared for analysis as previously described. The results are shown in Table IV.

TABLE IV

NICOTINIC ACID IN WHEAT BRAN

Method	Original extract	Oxidized extract
	μg/g	μg/g
Bina, Thomas, and Brown	140	138
Snell and Wright	157	147
Aniline method	256	137

The chemical methods showed a wide difference in results on the original extract of wheat bran, but give the same values on the oxidized extract. The microbiological values were slightly lowered by the oxidation treatment, but the difference was so slight as to be within the range of experimental error of the method as applied to material of this potency. The difference in values by the chemical and microbiological methods on the oxidized extracts was also slight. The values are of the same order of magnitude and are in the 5% range.

Milk powder: A sample of commercial skim-milk powder, when subjected to the same treatment as previously described, gave the values before and after oxidation that are shown in Table V.

TABLE V

NICOTINIC ACID ANALYSES OF MILK POWDER

Method	Original extract	Oxidized extract
	μg/g	μg/g
Bina, Thomas, and Brown	14	12.5
Snell and Wright	10.5	10.5
Aniline method	20.3	14.5

The values for milk powder showed close agreement by the microbiological and the Bina, Thomas, and Brown method on the original and oxidized extracts but not where aniline was used. The results by the chemical methods were similar to the values obtained in our previous publication but unfortunately they were erroneously reported as milligrams per pound.

Removal of Peroxides from Oxidized Extracts

It is essential that excess peroxides be removed from the oxidized extract prior to color development, in the chemical procedures. We find that even traces of peroxides render the color complex unstable for accurate reading. The aromatic amines used in the chemical procedures are attacked by excess amounts of the oxidizing agents to the degree in which they are present.

We have used two procedures for the satisfactory removal of the excess peroxides. After treatment of the extracts with hydrogen peroxide in acid solution as previously described, the extract should be evaporated to dryness on the steam bath, the residue redissolved in distilled water, neutralized, and again treated with hydrogen peroxide to complete the oxidation. When this solution is evaporated to dryness and the acids neutralized, the solution is usually suitable for colorimetric development. The degree of oxidation appears to be affected by the amount of oxidizable material the sample contains.

A second procedure involves the use of sodium bisulfite as the reducing agent to destroy the excess peroxides. The peroxide-treated extract, after removal of most of the hydrogen peroxide by boiling, is titrated with a 20% solution of sodium bisulfite, using starch iodine outside indicator. This procedure produces sulfates which are precipitated by barium chloride and removed in the centrifuge.

The use of Lloyd's reagent, as described by Dann and Handler (1941), is also applicable for this purpose. We find, however, that losses may occur in this procedure due to the various precipitates and manipulations involved. This is especially noticeable in the microbiological procedure where consistent results were not always obtained when this modification was employed. The presence of small amounts of peroxides does not affect the microbiological determination and their removal is not required when this procedure is used.

Influence of the Hydrogen Peroxide Treatment on Nicotinic Acid

Two hundred micrograms of nicotinic acid was treated according to our regular procedure with hydrogen peroxide in alkaline solution by boiling for 15 minutes and subsequent removal of excess peroxide. Determinations made on this oxidized solution gave recoveries of

99.25% and 107%, respectively, when determined by the chemical procedures. There was no difference in the recoveries of added nicotinic acid between the oxidized and unoxidized samples by the microbiological method. This range was from 80% to 120%.

Discussion and Summary of Results

The use of oxidizing agents to remove interfering compounds encountered in the determination of nicotinic acid shows that the microbiological method of Snell and Wright (1941) is not specific for nicotinic acid and that substances other than nicotinic acid influence the production of acid used as the measure of the test. The data obtained on the same extracts before and after treatment with hydrogen peroxide show that original extracts of whole wheat contain two types of compounds which are measured as nicotinic acid. The two types of compounds differentiate themselves chemically in the fact that one is readily oxidized and loses its properties as a chromogen, while the second type is stable to the same oxidizing reagents and continues to be reactive in the oxidized extract.

It is of interest to note that this readily oxidized chromogen is shown to differ chemically from nicotinic acid by the difference in solubilities in ethyl acetate. This difference in solubility permits the separation of this chromogen from the chromogen produced by nicotinic acid in the König reaction in which *p*-aminoacetophenone is used as the aromatic amine. The fact that the same values were obtained on the original and oxidized extracts where ethyl acetate was used shows the efficiency of this solvent in the separation of nicotinic acid from this type of interference.

The agreements and disagreements between the values by the microbiological and chemical methods on the materials we have discussed are not uniform but depend on the type of material investigated. No doubt a large part of this is due to the variability in the treatment and methods used. The oxidation treatment with hydrogen peroxide is subject to variation in the removal of chromogens from the extracts as time and temperature are important factors in the oxidation. The microbiological and chemical methods are subject to wide variations in results. Results by the microbiological method have been shown by other investigators to vary considerably according to the method of preparing the extract. This fact was amply verified by the values reported by five different laboratories on the same collaborative yeast sample by the microbiological method in which values were obtained ranging from 130 to 393 μg per gram. We avoided this difficulty in the experiments reported in this paper by supplying the same extracts for microbiological analysis as we used for chemical analysis.

The agreement between the results by the microbiological and chemical methods, on the oxidized extract of whole wheat in acid solution, is not as significant to us as the disagreement in the results obtained with alkaline oxidation. It is evident that oxidation treatment in the acid solution was more complete than in the alkaline solution as shown by the chromogen content in the oxidized extracts. The fact that the alkaline treatment produced a stimulant which increased the microbiological values not due to a chromogen in the König reaction, is of more significance.

The chromogens removed from the wheat germ extracts by hydrogen peroxide, which affected the aniline procedure in determination, did not affect the microbiological values. The fact that approximately the same values were obtained on the original and oxidized extracts of wheat germ substantiates our conclusion that nicotinic acid is not destroyed by oxidizing agents. The difference in values obtained for nicotinic acid by the chemical methods and the microbiological procedure on this wheat germ extract is too great to be ascribed to experimental error and represents a fundamental difference in the methods. Repeated experiments were made by each of the methods and consistent values of the same order were obtained. The value of 35 μg per gram, which was obtained by the microbiological method, agrees very closely with the average of 34 μg per gram reported by Elvehjem and co-workers (1942) for wheat germ by this method. Our experiments naturally were not made on the same wheat germ they used. The 56 and 53 μg per gram obtained by us on the original and oxidized extracts agree very closely but at a higher range than the microbiological values. The oxidized extract by the aniline method also was in this range.

It is apparent that the chemical and microbiological methods do not specifically measure the same material in wheat germ extracts. The results by dog bioassay, as well as the chemical properties of nicotinic acid, support the higher values obtained by the chemical methods for wheat germ extracts.

Teply, Strong, and Elvehjem (1942) report higher values for nicotinic acid on whole wheat than on wheat germ. In our experiments this is not confirmed by either the chemical or microbiological method when the determinations are made on the oxidized extracts. The values they obtain for whole wheat, more than twofold greater than our values, would appear to be due in part to material not possessing the chemical properties of nicotinic acid. Our microbiological value for wheat germ, which agrees closely with their result by that method, is only 66% of the value we obtained by the chemical procedure for

the same wheat germ. The low values given by the microbiological methods for wheat germ are not explainable by the chemical properties of nicotinic acid, since this method accounts for only a portion of the non-oxidizable chromogen contained in the extract. It would appear, therefore, that the microbiological method is subject to inhibiting as well as accelerating interferences.

The question of acid, alkali, and aqueous extractions and the production of hypothetical precursors of nicotinic acid which are then converted into nicotinic acid by the alkali or acid methods was encountered in this work. Oxidation shows that the end product producing the interference is not a chromogen and does not possess this property of nicotinic acid. It would appear that material responsible for this growth stimulant in the microbiological method is not the same as the interfering chromogens encountered in the chemical methods.

The separation of the nicotinic acid color complex with ethyl acetate as a supplement to the *p*-aminoacetophenone reaction is shown to be applicable and efficient as a method for the elimination of interfering chromogens characteristic of cereal products. The extraction readings of the nicotinic acid color complex in the ethyl acetate solvent are only approximately one-third the value of the same concentration when read in the aqueous phase. The only difference is that the amount of sample used is larger when ethyl acetate is employed than would be required if it were possible to make the readings on the aqueous phase.

No satisfactory blank has been developed for the use of aniline in the determination of nicotinic acid in cereal products where interfering substances are encountered. In the determinations in which this amine was used, we employed blank values in which the amine was added to the sample. We find this blank eliminates color produced by compounds other than nicotinic acid which are known to be present. The color that is eliminated by this type of blank varies with the material used and is not produced by nicotinic acid as a result of the König reaction. A comparison in values obtained for nicotinic acid when the aniline blank is used, as we employ it, with a reagent and material blank as employed by other investigators, is shown on a whole wheat extract. With aniline in the sample, we get a blank value of 0.055 and an apparent nicotinic acid content of 56.7 μg per gram. This same solution gave 59.6 μg per gram when the reagent and material blank value were used. Aliquots of this extract were oxidized and similar analyses were made on the oxidized extracts. The blank value on the oxidized extract containing aniline had been reduced to 0.008, giving a nicotinic acid content of 25.1 μg per gram, a value in good

agreement with the results obtained by the other methods on this material. This same extract showed 12.7 μg per gram when the material and reagent blank value was employed, indicating that cyanogen bromide is not a part of the blank value.

The production of chromogens and growth stimulants by alkaline hydrolysis in the estimation of nicotinic acid gives values that are compatible neither with the chemical properties of nicotinic acid, nor with the results obtained by animal experimentation, nor with experience encountered when this material is used in the diets of pellagrins. Andrews, Boyd, and Gortner (1942) show values for nicotinic acid varying more than two-fold on whole wheat extracts where the only difference in treatment is the presence of 0.25% NaOH for five minutes at room temperature when using the microbiological method, but could get no increase when wheat germ was so treated. Wheat germ shows to a much better advantage as a part of the diet in the treatment of pellagra than whole wheat, yet these authors, as well as Teply, Strong, and Elvehjem (1942), get approximately twice the value for nicotinic acid in whole wheat as they do in wheat germ when tested by the microbiological method. Schaefer, McKibbin, and Elvehjem (1942) could get no growth response from 500 g of whole wheat, assaying 5.5 mg per 100 g by the microbiological method, when fed to a dog, and were forced to add nicotinic acid before they could obtain growth. Their result shows that the amount of nicotinic acid contained in whole wheat is too small to be in the range of accurate dog bioassay. The supplemental value those authors obtained after administering nicotinic acid was undoubtedly due in part to the supplemental value of other vitamins in the whole wheat when fed in so large amounts as they used. This is true of bioassay values with other vitamins, where mixtures of yeast and wheat germ give higher values for thiamin and riboflavin than when bioassayed separately under similar conditions.

The value of whole wheat as a source of nicotinic acid and whole wheat bread as a vital factor in the prevention of pellagra has been greatly overestimated. This overestimation has come about as a result of chromogens encountered in the chemical procedures and non-nicotinic acid growth stimulant measured in the microbiological method.

The data fail to confirm the presence of any hypothetical precursor of nicotinic acid as proposed by the proponents of the microbiological and of some of the chemical methods in order to explain their high values for nicotinic acid in cereal products, but corroborate the low results obtained by Kodicek (1940) on dog bioassay, and the chemical values obtained by Thomas, Bina, and Brown (1942).

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THE PREDICTION OF LOAF VOLUME OF HARD RED SPRING WHEAT FLOURS FROM SOME PROPERTIES OF MIXOGRAMS [1]

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The estimation of dough properties from micro-recording-mixer curves has received marked attention in relation to the prediction of baking strength. Such curves made from hard red winter wheat flours have been discussed by Swanson and his associates. Swanson (1939, 1941), Swanson and Andrews (1942), and Johnson, Swanson, and Bayfield (1942) have critically examined these curves and their properties in relation to varietal quality evaluation of Kansas wheats.

Larmour, Working, and Ofelt (1939) showed that recording-dough-mixer curves differed in character at various protein levels and that curve height increased with protein content. Sandstedt and Ofelt

[1] Published with the approval of the Director of the Experiment Station.

(1940) studied the baking properties of flours diluted to a common protein content with wheat starch, and inferred therefrom that the mixing behavior of flours should likewise be compared at an uniform protein level. These workers (Ofelt and Sandstedt, 1941) later carried out an investigation employing a National micro recording mixer. Predetermined absorptions were used in mixing and the protein content was reduced in decrements of 1.0%. The curves obtained were very similar to those yielded by flours with the same naturally occurring protein content and showed a like tendency to decrease in height

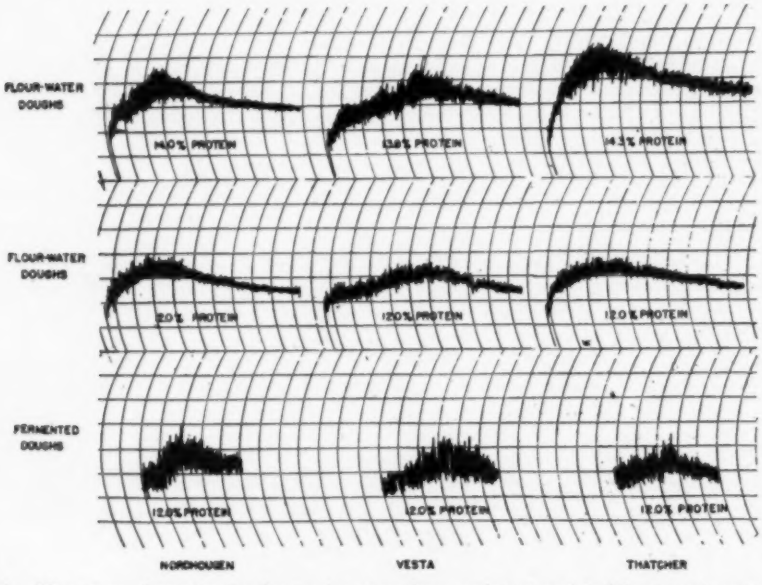

Fig. 1. Mixer curves from three hard red spring wheat flours made at the original flour protein content, at a constant flour protein level, and following 3 hours of fermentation.

and flatten out with lower protein content. Mixing time was found to be a varietal rather than a flour-protein-content characteristic.

Harris, Sibbitt, and Banasik (1942) published the results of a preliminary study made with the object of predicting the loaf volumes of North Dakota spring wheat flours from mixer-curve properties. The curves were obtained from flour doughs which had been previously mixed and fermented in the usual manner for 3 hours. The flours were diluted to an uniform protein content of 12.0% (13.5% moisture basis) with hard red spring wheat starch. Replicates of the doughs were baked on the same day the mixer tests were run. Following fermentation one aliquot of the original dough was placed in the mixer bowl and a 5-minute mixing curve obtained, while the remaining portion was panned and baked. Figure 1 shows curves obtained from three wheat

flours at the original protein content, at an uniform protein level of 12.0%, and after 3 hours of dough fermentation. Very significant changes in curve properties are evident in going from the flour-water curves at the original flour protein content through the curves made at an uniform protein level to the fermented dough results. The greatest change in the curve characteristics are shown in the mixings made with the fermented doughs. Reducing the protein content to a constant level lowered the height of the curves and decreased the band width. Fermentation, on the other hand, markedly increased band width and tended to obscure varietal differences. It was found that after 5 minutes of mixing, these fermented doughs broke down badly and showed no probability of further mixing yielding information in regard to wheat quality.

It was felt that the records made with fermented doughs might more truly represent the physical changes taking place in the dough and be more indicative of loaf volume than curves obtained from flour-water doughs without fermentation. Thirteen hard red spring wheat flours were accordingly diluted with wheat starch to a constant protein level of 12.0% and mixed in the Hobart, equipped with special dough hooks, to a dough of normal consistency and treated as described. The malt-phosphate-bromate baking formula was used with 50 g of flour.

The following curve properties appeared to be related to the loaf volume of the flours investigated: (1) the magnitude of dough development angle, (2) the magnitude of the angle of decline of the curve toward the vertical, (3) time to reach peak dough development, and (4) height of the curve from the base line. From these factors an equation was formulated for the prediction of loaf volume.

The diagrams used in calculating a single-figure score for two flours of radically different baking strengths are shown in Figure 2. To construct the diagram a line AD is drawn through the center of the initial width of the band parallel to the horizontal axis. A line BD is then drawn tangent to the declining portion of the curve, cutting the base line AD at D. The center of the peak of the curve is next found by drawing the line BC through the midpoint of the distance representing the stability of the dough, or line of mixing tolerance. From point B, where BD and BC intersect, a line AB is drawn tangent to the rising portion of the curve, meeting the base line at A.

The different measurements made were taken in millimeters. The magnitude of α (dough-development angle) can be expressed by the cosine AC/AB. As α decreases, the cosine increases. Similarly, the magnitude of β (or angle of decline of the curve) can be expressed as the sine CD/BD. The linear value AC is related to loaf volume, and, accordingly, the functions of the two angles must be multiplied by AC.

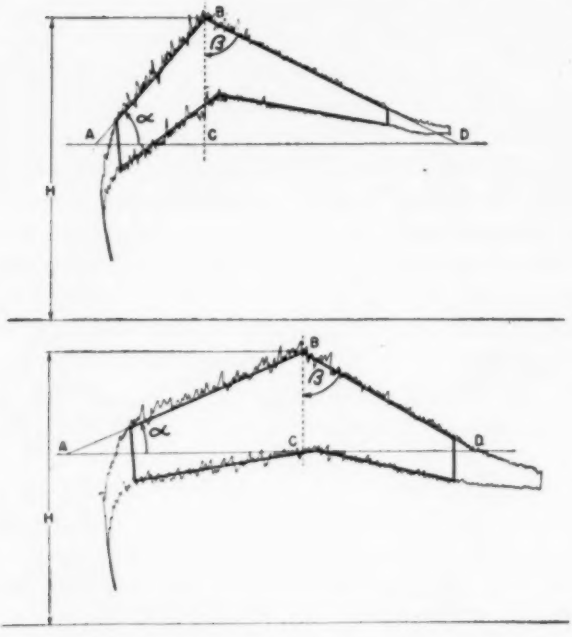

Fig. 2. Diagrams used for computing calculated loaf volumes from mixer curves of fermented doughs made from flours of different baking strength. ABOVE: Actual loaf volume, 95 cc; calculated volume, 98. BELOW: Actual loaf volume, 150 cc; calculated volume, 148.

$$\text{Loaf volume} = K(\cos\alpha \times \sin\beta)AB \times \text{height}$$
$$= K(AC/AB \times CD/BD)AB \times \text{height}$$
$$K = 4.24.$$

The height of the curve measured from the base line is also included in the formula. Differences in the magnitude of the angles α and β, as well as in the linear values, can be readily distinguished between the diagrams for the two flours. The constant K was evaluated from curves made with flours of known loaf volume and was found to have a value of 4.24 in the present instance. New constants would, no doubt, have to be determined in work with other types of wheat, as well as with wheat from other crop years. The computed single-figure score was found to be highly correlated with loaf volume, a coefficient of +.943 being obtained between the two variables. Similar relationships were also found in the case of studies made with starch-gluten blends at a constant protein level. In view of these results the following investigation was undertaken to obtain further information regarding the relationships between the curve properties and loaf volume at an uniform protein level.

Experimental

Thirty hard red spring straight-grade flours experimentally milled from the 1941 North Dakota crop were used for this study. These wheats consisted of seven different varieties grown at four stations and an additional variety grown at one station only. One commercially milled spring wheat flour was also included. The wheat protein varied from 13.6% to 16.4% and the flour protein from 11.9% to 15.7%, while flour ash ranged from 0.34% to 0.52%. The loaf volumes of the undiluted flours ranged from 105 cc to 200 cc with an average volume of 155 cc. To obviate the effects of protein content differences upon the mixogram characteristics and to confine the investigations to a study of *quality* variability, the flours were diluted with hard red

TABLE I

COMPARATIVE MIXOGRAM DATA FROM HARD RED SPRING WHEAT FLOUR DOUGHS FERMENTED FOR 3 HOURS WITH CALCULATED AND ACTUAL LOAF VOLUMES. FLOUR PROTEIN LEVEL 12.0%

(Data arranged in order of increasing loaf volume)

Lab. No.	Variety	Cosine α	Sine β	Length AC	Height	Loaf volume	
						Calculated	Actual
				mm	*mm*		*cc*
47	Thatcher	0.74	0.91	3.2	8.1	98	95
74	Rival	0.80	0.95	3.3	7.2	96	95
48	Rival	0.69	0.89	3.3	8.7	108	105
250	Rival	0.72	0.93	3.4	8.3	111	107
57	Nordhougen	0.70	0.93	3.3	8.5	111	110
93	NN 2829	0.84	0.94	3.8	7.6	115	110
69	NN 2822	0.84	0.89	3.6	8.2	111	112
70	NN 2829	0.75	0.94	3.3	8.1	106	112
54	Vesta	0.68	0.92	3.4	8.7	115	115
256	Vesta	0.76	0.89	3.7	8.3	116	115
265	NN 2829	0.84	0.94	3.8	7.4	112	117
81	Vesta	0.76	0.91	3.8	8.3	122	120
259	Nordhougen	0.74	0.90	3.9	8.7	130	124
59	Regent	0.69	0.92	3.4	9.3	123	125
264	NN 2822	0.84	0.94	3.7	8.1	120	126
235	Thatcher	0.94	0.92	4.6	7.4	133	135
92	NN 2822	0.81	0.92	4.2	8.4	138	137
240	Vesta	0.78	0.90	4.3	8.7	143	137
61	Haynes	0.79	0.88	3.7	9.2	127	140
249	Thatcher	0.86	0.95	4.3	7.9	140	140
261	Regent	0.80	0.93	4.3	8.4	142	140
280	Commercial flour	0.84	0.83	4.6	8.4	136	140
247	NN 2829	0.91	0.94	5.0	7.4	147	142
236	Rival	0.84	0.95	4.6	8.0	148	145
246	NN 2822	0.94	0.95	4.7	7.6	144	146
73	Thatcher	0.81	0.90	4.3	9.0	149	148
84	Nordhougen	0.83	0.94	4.3	8.6	147	148
244	Regent	0.78	0.89	5.6	8.5	141	150
242	Nordhougen	0.81	0.93	4.2	8.3	138	158
86	Regent	0.73	0.91	4.3	9.4	156	160

spring wheat starch to an uniform level of 12.0% (13.5% moisture basis). This starch was prepared by the method described by Harris and Sibbitt (1941) and contained 0.5% protein.

The doughs were mixed in the Hobart as described, 50 g of flour being used with the malt-phosphate-bromate formula, and absorption as required for optimum consistency. The amount of water needed varied somewhat among the various samples despite the fact that they were on the same protein level. The doughs after mixing were divided into two equal portions by weight and fermented 3 hours with the customary punches. At panning time one dough was panned and baked, while the other was placed in the mixograph and a 5-minute mixogram obtained.[2]

From the mixograms the various data were derived by the method described. These values are shown in Table I. Although the flours contained the same quantity of protein, large variations in loaf volume were obtained. One sample of Thatcher produced the smallest loaf with three Rival samples next. This variety has shown much promise in baking quality, but was rather disappointing in baking performance in 1941. Regent, a Canadian wheat, and Nordhougen gave very satisfactory results by this method of baking. Differences are evident among all the data shown in the table, although little consistent change in the values with increase in actual loaf volume is to be noted, except for the length of AC and the calculated loaf volume. The correlation coefficients between these values and the loaf volume are presented in Table II.

It will be noted that the agreement between the computed and actual loaf volumes is very good indeed, with a correlation coefficient of +.951 between the two sets of values. The corresponding means and standard deviations were 127.4, 128.5, 16.5, and 17.9 cc, respectively. It is quite evident that loaf volume may be predicted from the calculated values with an accuracy entirely satisfactory for practical purposes, the prediction equation being loaf volume = 1.0 × calculated value − 3.3. One factor, the length of the line AC, which enters into the calculation of the single-figure value, is likewise rather highly correlated with loaf volume. This coefficient is, however, significantly lower than the relationship between single figure score and loaf volume and has a correspondingly greater error of estimate. This error for the former method of prediction by the single figure score is 5.6, whereas by using the line AC it is 10.7. The other variables do not show a suf-

[2] Mixograph and mixogram refer respectively to the National recording-micro-mixer and the curve produced thereby. This nomenclature was suggested by Dr. E. G. Bayfield and will be used in the following discussion.

ficiently high relationship to serve as factors for predicting loaf volume, although they are significantly correlated with it.

Scatter diagrams, with regression of loaf volume on the values obtained from the mixogram data, are shown as Figures 3 and 4. Figure 3 presents the relationship between calculated and actual loaf volume, while Figure 4 shows the relationship between the length of line AC

TABLE II

CORRELATION COEFFICIENTS CALCULATED FROM THE MIXOGRAM AND BAKING DATA
(Significant coefficients in bold type)

Variables correlated		
x	y	r_{xy}[1]
Loaf volume calc., 3 hrs	Loaf volume actual, 3 hrs, cc	**+.951**
Length AC, 3 hrs, mm	Loaf volume actual, 3 hrs, cc	**+.803**
Cosine angle α, 3 hrs	Loaf volume actual, 3 hrs, cc	**+.413**
Sine angle β, 3 hrs	Loaf volume actual, 3 hrs, cc	−.060
Height, 3 hrs, mm	Loaf volume actual, 3 hrs, cc	+.280
Loaf volume calc., 2 hrs	Loaf volume actual, 2 hrs, cc	+.340
Cosine angle α, 2 hrs	Loaf volume actual, 2 hrs, cc	−.155
Sine angle β, 2 hrs	Loaf volume actual, 2 hrs, cc	**−.644**
Length AC, 2 hrs, mm	Loaf volume actual, 2 hrs, cc	+.304
Height, 2 hrs, mm	Loaf volume actual, 2 hrs, cc	+.350
Loaf volume calc., 3 hrs	Loaf volume actual, 2 hrs, cc	**+.483**
Length AC, 3 hrs, mm	Loaf volume actual, 2 hrs, cc	+.264
Length AC, 3 hrs, mm	Length AC, 2 hrs, mm	+.185
Loaf volume actual, 3 hrs, cc	Loaf volume actual, 2 hrs, cc	**+.448**
Loaf volume calc., flour-water doughs	Loaf volume actual, 2 hrs, cc	+.047
Loaf volume calc., flour-water doughs	Loaf volume actual, 3 hrs, cc	+.246

[1] Value of r_{xy} at 5% point = 0.362.

in millimeters and actual loaf volume. The higher degree of interdependence between the variables in Figure 3 is clearly evident, with correspondingly greater accuracy in the prediction of loaf volume.

In view of the satisfactory results yielded by the 3 hour fermentation procedure, it was thought advisable to determine the effect of shortening the fermentation period of the doughs previous to remixing in the mixograph. If satisfactory results could be obtained in a shorter time, the value of the method would be materially increased. Periods of 2, 1, and 0 hours were accordingly used on this series of flours, the same baking formula being employed throughout. In addition, one complete run was made with a flour-water dough as customarily employed for mixograms. Measurements as before were taken from each set of curves thus obtained.

The individual results from these shorter and no fermentation times are not shown, but the correlation coefficients obtained for the 2 hour period are presented in Table II. Correlations between calculated and actual loaf volumes for the flour-water doughs are also given. The

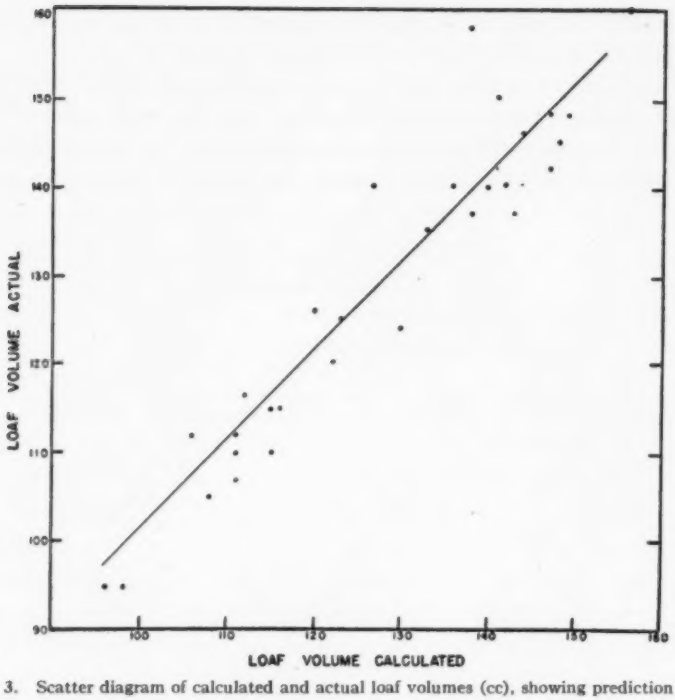

Fig. 3. Scatter diagram of calculated and actual loaf volumes (cc), showing prediction line.

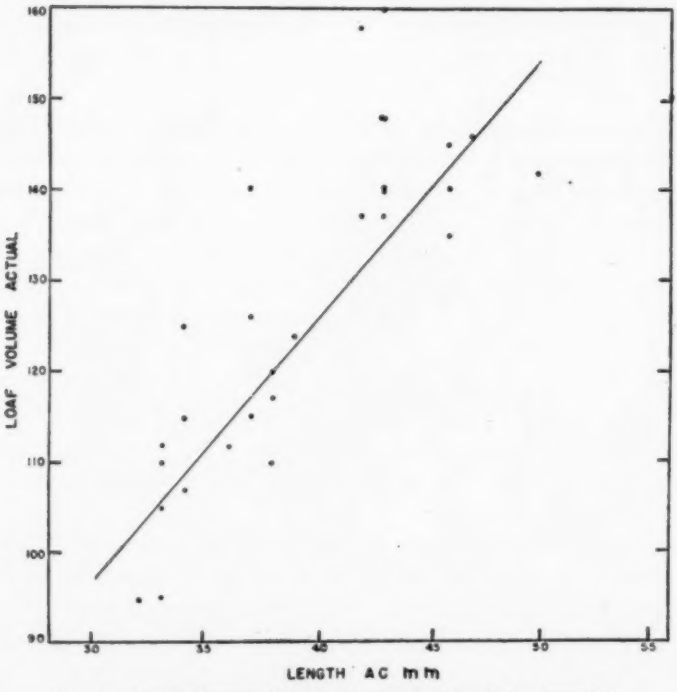

Fig. 4. Scatter diagram of length of *AC* and actual loaf volume (cc) with prediction line for loaf volume from *AC*.

only variable in this group which appeared to be correlated to any appreciable degree with loaf volume was the sine of the angle β in the two-hour series. This is a negative relationship, but is not high enough to be of any practical importance for the purpose of predicting loaf volume. No significant relationships between mixogram properties or calculated score and actual loaf volume were secured from the 1- and 0-hour fermentations. Apparently the 3 hours of fermentation had an effect upon the mixograms which brought out the relationships among the various factors required for computing a satisfactory single-value score. Mean loaf volumes were higher in the 2-hour fermentation studies, being 154 cc for the calculated values and 158 cc for the actual bakings. The length of AC was also greater, averaging 4.6 as compared with 4.0 in the 3-hour doughs. Standard deviations for loaf volume were 14.3 and 14.9, respectively.

Table III presents the relationships found in mixogram data obtained from flours with protein contents not adjusted to a common level

TABLE III

CORRELATION COEFFICIENTS OBTAINED BETWEEN THE DIFFERENT VARIABLES, AT ORIGINAL FLOUR PROTEIN CONTENTS

(Significant coefficients in bold type)

Variables correlated		
x	y	r_{xy}[1]
Length AC, mm	Loaf volume actual, cc	**−.446**
Height, mm	Loaf volume actual, cc	+.324
Calculated loaf volume	Loaf volume actual, cc	−.345
Flour protein, %	Loaf volume actual, cc	**+.815**
Length AC, mm	Flour protein, %	−.334
Height, mm	Flour protein, %	+.360
Sine angle β	Flour protein, %	−.140
Cosine angle α	Flour protein, %	−.289

[1] Value of r_{xy} at 5% point = 0.362.

by dilution with starch. Curve properties were correlated with loaf-volume and flour-protein content. Flour protein content was rather closely related to loaf volume, while AC had a smaller but still significant correlation with the latter variable. The coefficient between curve height and flour protein barely significant, while the other curve characteristics were not.

Table IV shows the comparative effects of the different treatments upon mixogram properties. In the flour-water mixes the higher protein content affected only height of the curve, while the other values shown remained practically unchanged. The effect of adding the baking ingredients was to lengthen AC and increase the calculated score. Fermentation decreased AC, and possibly the height; no consistent

TABLE IV

AVERAGE VALUES OF VARIOUS MIXOGRAM PROPERTIES OBTAINED FROM THE DIFFERENT SERIES OF DOUGHS

Fermentation (in hours)	Flour-water doughs		Malt-phosphate-bromate-formula doughs			
	0[1]	0	0	1	2	3
Length AC, mm	4.2	4.7	7.7	3.0	4.6	4.0
Height of curve, mm	9.3	8.2	9.1	9.1	8.5	8.3
Cosine α	0.79	0.77	0.82	0.67	0.84	0.79
Sine β	0.91	0.91	0.89	0.92	0.91	0.92
Calc. loaf volume	151	146	260	107	154	127

[1] Measurements made at original flour protein content; all others at 12.0% protein level (13.5% moisture basis).

changes in the other values were found. As AC appeared to be the most important in relation to loaf volume of any of the mixogram values studied, in addition to being more sensitive to dough-treatment changes, it was decided to examine the effect of wheat variety upon this variable. Table V presents the results obtained.

The long dough development period of Vesta, as registered by the length AC, is clearly evident, especially with the baking formula and no fermentation. Rival is next, with No. 2822 having the shortest

TABLE V

COMPARATIVE LENGTHS OF AC OBTAINED FROM THE DIFFERENT WHEAT VARIETIES STUDIED

Fermentation (in hours)	Flour-water doughs		Malt-phosphate-bromate formula doughs				Average
	0[1]	0	0	1	2	3	
	mm	mm	mm	mm	mm	mm	mm
NN 2822	3.5	3.1	4.9	2.6	4.4	4.0	3.8
Nordhougen	4.1	4.6	6.3	2.1	4.7	3.9	4.3
NN 2829	4.1	3.5	6.8	2.1	4.7	4.0	4.2
Regent	3.7	4.5	6.6	2.7	4.4	4.4	4.4
Vesta	5.5	7.3	12.3	4.8	4.6	3.8	6.4
Thatcher	4.3	4.7	6.9	3.6	4.8	4.1	4.7
Rival	4.4	5.1	9.3	2.9	4.8	3.6	5.0

[1] Measurements made at original flour protein content; all others at 12% protein level (13.5% moisture basis).

period. It seems to the authors that this particular flour property should be measured by the use of a baking formula rather than a flour-water dough, inasmuch as this treatment corresponds to the way in which the flour will actually be used. There was some evidence of an effect of location of growth upon AC.

Comparative mixograms obtained by progressively reducing fer-

mentation time from 3 to 0 hours are shown in Figure 5 for seven hard red spring wheat varieties. Curves made from flour-water doughs are also included. Mixograms made from doughs containing the baking ingredients were more informative than flour-water dough curves. Band width was definitely increased by these ingredients and varietal characteristics were brought out more clearly. It would therefore seem

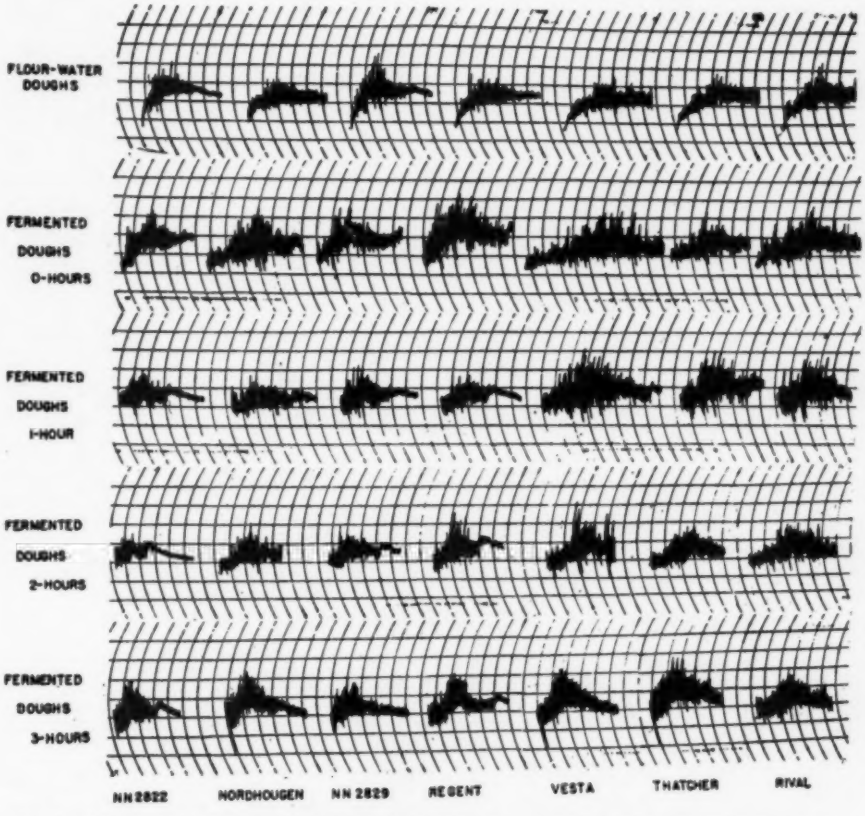

Fig. 5. Mixograms of 7 flours milled from different wheat varieties made from flour-water doughs and from yeasted doughs fermented for various lengths of time.

preferable to use the malt-phosphate-bromate or some other comparable formula rather than distilled water alone in making mixograms. Dough fermentation, as previously pointed out, decreased dough development time and tended to obscure varietal differences. Sample 2822, which has a short mixing time, was least affected by fermentation. The long dough-development time required by Vesta and Rival is clearly evident, especially with the nonfermented malt-phosphate-bromate doughs. The comparatively short development time of sam-

ple No. 2822 is also apparent. Characteristic changes induced by the lengthening of fermentation period may be discerned.

The weakest point of the method described for calculating loaf volumes from the mixogram data lies in determining the midpoint of certain of the lines involved. For instance, it is sometimes difficult to determine the exact center of the rising curve at the point of initial width. Another debatable point is the precise length of the line representing dough stability.

The data presented in this paper, while showing the utility of mixogram properties in predicting flour loaf volume under a specified baking formula at a stated protein level, should not be interpreted as leading to the conclusion that the baking test may be largely superseded by these curves made from fermented doughs. The present work, however, does suggest, in the authors' opinion, the replacement of a portion of the actual bakings with hard red spring wheat varieties by mixograms, with consequently less time and labor involved in the measurement of baking strength. Further investigations are in progress regarding the effect of different baking formulas upon mixogram properties.

Summary and Conclusions

Data computed from certain properties of mixograms made from 30 samples of eight varieties of hard red spring wheats were correlated with the loaf volumes obtained following 2- and 3-hour fermentations. Similar data yielded by flour-water doughs were also examined. The curve properties considered were as follows:

1. Cosine of the angle of dough development, or angle which the ascending portion of the curve makes with the horizontal.
2. Sine of the angle of decline of the curve, or angle which the descending portion of the curve makes with the vertical drawn through the curve peak.
3. Length in millimeters of the horizontal line between the point where it is cut by the tangent to the ascending curve and the vertical line dropped from the midpoint of the distance representing dough stability.
4. Height in millimeters of the peak of the curve from the base line.

These factors were combined in an equation to yield a value indicative of the loaf volume of the flour under examination.

A very high relationship was found between the calculated and actual loaf volume when the mixograms were made from doughs containing the usual baking formula and mixed and fermented 3 hours in the same manner as for baking bread. This correlation was large enough to render possible the prediction of actual loaf volume by the equation. The length of line between the tangent to the rising curve and the vertical through the midpoint of dough stability was related to a lesser degree with loaf volume. The other curve properties considered had little or no relationship to loaf volume.

When the doughs were fermented for shorter periods than 3 hours, the relationship between actual and calculated loaf volume disappeared. The values calculated from curves made from doughs following 2, 1, and 0 hours of fermentation were found to have no significant correlation with the actual loaf volumes of the flours obtained from either 3 or 2 hours of fermentation.

Mixograms made from doughs containing the malt-phosphate-bromate-formula ingredients showed wheat varietal differences more distinctly and were easier to interpret in terms of curve properties studied. Band width was increased.

Fermentation consistently decreased time of dough development, and varietal differences were shown in dough-development time. Curves made from doughs with the malt-phosphate-bromate baking formula at 3 and 0 hours of fermentation appeared to be the most useful in determining varietal differences in mixing time and baking strength.

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STUDIES ON TREATING WHEAT WITH ETHYLENE. I. EFFECT UPON HIGH MOISTURE WHEAT [1]

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and

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As a rule freshly harvested wheat does not germinate well, nor does flour made therefrom bake well. A period of storage is necessary to bring either of these functions to the optimum level. Previously reported experiments (Balls and Hale, 1940) have shown that flour from wheat harvested while somewhat immature underwent an almost immediate improvement in baking quality, and likewise an increase in germination capacity of the grain, when exposed to air containing traces of ethylene. On the other hand, the flour from immature wheat (before treatment with ethylene) was not improved by subsequent exposure to gas, although eventually it improved, like other fresh flour, on lengthy storage. Thus it seems that the improvement in the baking quality of the flour is brought about by the effect of the ethylene on the whole grain. It is accompanied, as shown below, by a temporary rise in carbon dioxide output (frequently referred to as "respiration"). Furthermore the effect is not particularly pronounced on grain already aged in storage.

It appears that the effect of ethylene on grain is similar to that observed by Denny (1924) on lemons, by Chace and Church (1927) and Chace and Sorber (1928) on many other fruits, and by Chace and Sorber (1936) on walnuts. The chemistry of these effects is not yet known; in practice they amount to an acceleration of what is generally meant by "ripening."

On the theory that ripe grain may keep better than unripe grain of like moisture content, the possibility is indicated that the treatment of wheat with ethylene might materially increase its keeping qualities in storage. Experiments on the storage of grain are, however, admittedly of little value unless the quantity of grain handled is sufficient to simulate conditions obtaining in practice. Such conditions cannot at present be obtained with certainty in experiments of laboratory scale. It was accordingly arranged through the cooperation of the Office of Experiment Stations and the Kansas Agricultural Experiment Station to make a relatively large scale experiment with wheat at

[1] Enzyme Research Laboratory, U. S. Dept. of Agr., Bureau of Agr. Chem. and Engineering. Contribution No. 81; Dept. of Milling Industry, Kansas Agr. Exp. Sta. Contribution No. 90.

[2] U. S. Department of Agriculture, Bureau of Agricultural Chemistry and Engineering.

[3] Department of Milling Industry, Kansas Agricultural Experiment Station. (Part of this work was done under Special Research funds.)

Manhattan, Kansas. The handling, milling, and testing of the grain, as well as the baking tests, were under the direction of E. G. Bayfield, and with the use of the facilities of the Kansas Agricultural Experiment Station. The results of these experiments on grain of relatively high moisture are reported in this paper.

It was found that when moist wheat (17.2% H_2O) was exposed to ethylene, its keeping quality was decidedly bettered as compared with similar but untreated wheat.

Experiments on High-Moisture Wheat

About 700 bu. of Tenmarq wheat with an average moisture content of 17.2% was harvested by a combine in the vicinity of Manhattan. The harvest (considering prevalent weather conditions) was about 6 days before the grain would ordinarily have been considered dry enough for combining. The wheat was brought at once to the Kansas State College mill and alternate loads were put into each of two bins. Wheat from each bin was then blended in filling the bins used for storage during the test. Two cylindrical metal bins were used approximately 6 feet in diameter and 14 feet in height. Three resistance-type thermometers were centrally installed in each bin, and so located that vertically they were equidistant from each other and from the top and bottom of the space occupied by the grain.

The wheat in one of the test bins received no immediate treatment. The wheat in the other bin was treated with ethylene. The gas was admitted to this bin as the grain was being filled in. The time required to put in the grain was 90 minutes, and ethylene was injected at the rate of one liter in 30 minutes making a total of 3 liters in a total space of approximately 11,000 liters, partly filled with wheat. The gas was admitted a foot or two above the level of wheat existing at the moment; and was thus rapidly mixed with the column of air in the bin through which the grain was falling.

During storage, temperature readings were made at regular intervals on the three pyrometers, respectively described hereafter as representing the temperature of the top, middle, and bottom of the bins. Both samples of wheat, but particularly the untreated sample, had to be cooled occasionally by moving over a separator. Samples for milling and baking tests were removed periodically.

Figure 1 shows the temperature in the bins. From these data, it may be seen that the untreated wheat began to heat in about three days, and continued to do so in spite of repeated passages over a separator. Long before the observations were discontinued this wheat had become bin-burnt and very musty, so that it was useless for milling into flour. Experiments showed that as little as 15%,

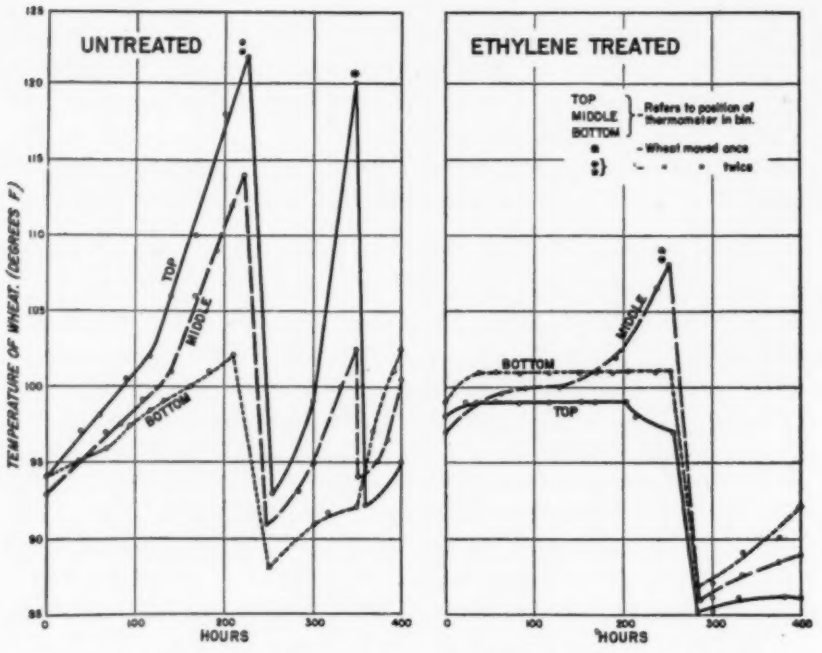

Fig. 1. Heating data during first 400 hours. Top, middle, and bottom represent relative positions of thermometers in the bins. The wheat in untreated bin was turned twice during first 400 hours. The wheat was passed twice over the separator during the first turning.

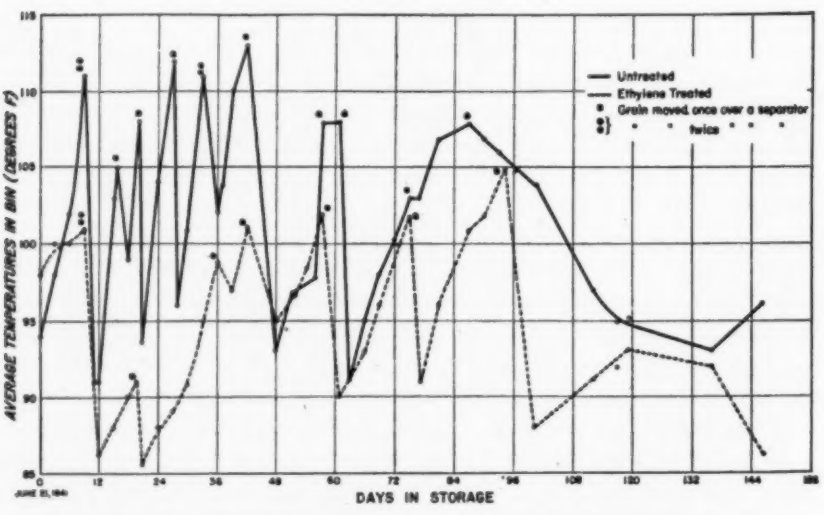

Fig. 2. Temperature of stored wheat (initial moisture 17.2%). The temperatures shown are the mean of the three simultaneous readings of the three thermometers located respectively in the top, middle, and bottom of the wheat.

blended in normal wheat when milled, imparted a musty taste to the flour and bread baked therefrom.

The treated wheat remained relatively quiescent for 9 days. It was moved, however, at the same time as the control grain on the ninth day. Subsequent observations showed that the treated wheat heated more slowly and required less moving to keep the temperature within safe bounds. Nevertheless, it could not have been stored safely without any moving at all. This was to be expected, because of the very high moisture content. The object of the experiment was achieved, however, in comparing the behavior of the two bins. It is apparent that the ethylene effect, while not enough to save such very moist wheat from damage over many months, did produce a marked decrease in the heating of the grain.

Germination and baking tests seem to bear out these conclusions. Germination tests (Table I) showed that 28% of the untreated wheat

TABLE I

EFFECT OF ETHYLENE ON GERMINATION OF WHEAT
(High-moisture wheat)

Bin No. 6 (untreated)			Bin No. 7 (treated)		
Date sampled 1941	No. days after filling bin	Germination [1]	Date sampled 1941	No. days after filling bin	Germination [1]
		%			%
6–23	2	64	6–23	2	78
6–24	3	86	6–24	3	86
6–25	4	96	6–25	4	60
6–26	5	82	6–26	5	84
7– 1	10	44	7– 1	10	74
7– 9	18	28	7– 9	18	80
9– 3	74	0	9– 3	74	0
10– 2	103	0	10– 2	103	0

[1] Germination tests made on November 28, 1941.

germinated after 18 days, while 80% of the treated wheat was still alive. In 74 days the grain in both bins was completely nonviable. In making these tests, samples of the grain were taken from the bins on the dates specified, exposed to fresh air, and kept thereafter in small containers at 32°F until tested.

One effect of the ethylene treatment in the wheat was an immediate increase in the CO_2 output of the grain (Table II). When considered in connection with the temperature chart, it does not seem likely that this increase could have been due to the development of microörganisms, but rather to a stimulation of metabolism in the seed. It is interesting to note that the excess carbon dioxide produced by the treated wheat would amount in the first five days to roughly 200 liters

more than that produced by the untreated grain. This is insignificant compared with the total of about 1250 liters produced by the treated wheat. If the air space is taken as a third of the volume of the grain, the maximum possible concentration of carbon dioxide would be about 34% in the case of the treated and 28% in that of the untreated

TABLE II

EFFECT OF ETHYLENE ON THE RESPIRATION RATE OF FRESHLY HARVESTED TENMARQ WHEAT

Interval after beginning of storage	Evolution of CO_2 at room temperature [1]	
	Wheat untreated	Wheat treated
hours	*mg/100 g of wheat/24 hrs*	
45 – 62.5	4.1	4.9
62.5– 87.5	3.1	5.2
87.5–111.5	2.9	4.3

[1] Determined on 1500-g samples by means of the apparatus of Truog (1915).

wheat. The leakage of gas from such bins is very considerable, but yet not immediate. It is obvious that in the case of leakage amounting to three fourths or more of the total CO_2, the concentration in the treated bin would still be significantly greater than that in the untreated. As a result of the ethylene treatment, the grain may therefore have been stored in an atmosphere containing a significantly higher concentration of carbon dioxide and for this reason it may have suffered a decrease in metabolic activity. This hypothesis will be investigated when opportunity permits.

Milling and Baking Results

Table III gives grain temperatures for various sampling dates together with analytical data for wheat and flour. The samples were normally milled on the day of sampling, the sample milled being taken from a 3 to 4 bushel lot drawn from the bin at each sampling. Only a few bushels remained in each bin at the time of the last two sampling dates, so that the bin residues were dried out somewhat. The high moisture content of the grain during the earlier phases of the experiment meant that no tempering of the grain was possible. This made milling difficult. During the latter part of the experiment, after the grain was damaged by more or less heating, milling was also difficult because maintaining normal flour ash levels and yields is practically impossible with such damaged grain.

Examination of the flour protein data shows that the wheat in the two bins was relatively uniform. In view of this, correlations of the

TABLE III

STORAGE AND ANALYTICAL DATA FOR TREATED AND UNTREATED SAMPLES

No.	Date sampled	Grain temp. °F			Test weight	Wheat moist.	Flour		
		Top	Middle	Bottom			Yield	Protein[1]	Ash[1]
					lb	%	%	%	%
UNTREATED—BIN 6									
993	6/23/41	97	95	95	57.5	17.1	67.5	11.5	0.56
995	6/24/41	99	97	96	57.0	17.0	68.2	11.5	.52
997	6/25/41	100	98	97	—	17.3	71.2	11.7	.54
999	6/26/41	102	99	98	56.7	17.0	70.6	11.7	.50
1005	7/ 1/41	122	114	102	53.8	17.6	69.0	11.4	.58
1007	7/ 9/41	95	99	101	—	17.0	68.2	11.4	.54
1011	7/15/41	107	105	101	—	16.1	67.8	10.8	.58
1015	7/22/41	103	108	104	—	16.5	69.6	11.5	.62
1019	8/ 5/41	120	118	110	53.4	16.5	68.5	11.4	.63
1023	8/19/41	110	105	103	—	15.9	68.5	11.3	.66
1027	9/ 3/41	101	103	102	54.9	14.6	72.0	11.4	.69
1030	10/ 2/41	—	—	—	53.7	15.0	70.0	11.6	.67
1955	11/ 7/41	100	100	89	—	14.9	68.3	11.4	.63
1961	12/15/41	discontinued			53.7	14.8	68.9	11.4	.61
1965	1/19/42				56.5	13.9	68.2	11.8	.71
2051	3/ 3/42				56.8	12.5	68.5	11.7	.68
ETHYLENE TREATED—BIN 7									
994	6/23/41	99	99	101	56.5	17.2	68.6	11.5	.57
996	6/24/41	99	100	101	56.7	17.4	69.6	11.5	.50
998	6/25/41	99	100	101	—	17.0	69.8	11.4	.53
1000	6/26/41	99	100	101	57.6	—	71.0	11.1	.51
1004	7/ 1/41	98	106	101	56.5	16.3	69.6	11.1	.54
1006	7/ 9/41	86	90	92	—	15.1	68.8	11.2	.51
1010	7/15/41	86	88	89	—	15.3	67.8	11.2	.52
1014	7/22/41	87	94	96	—	15.5	71.2	11.4	.59
1018	8/ 5/41	105	103	102	56.2	15.7	69.7	11.3	.56
1022	8/19/41	107	105	101	58.3	15.0	69.5	11.3	.59
1026	9/ 3/41	99	103	102	57.1	15.4	69.4	11.2	.58
1031	10/ 2/41	—	—	—	57.5	14.7	66.3	11.0	.58
1956	11/17/41	—	95	92	—	13.7	69.0	11.1	.58
1960	12/15/41	discontinued			57.5	13.6	68.8	11.1	.55
1964	1/19/42				58.5	14.1	67.7	11.4	.60
2052	3/ 3/42				57.8	10.8	68.7	11.4	.56

[1] Moisture basis 15%.

treatments, respectively, with test weight, moisture content, and number of times the wheat was turned are interesting. It is evident that the ethylene not only aided in reducing the grain temperatures but also produced a beneficial effect upon test weight and grain moisture content.

Table IV gives the baking data for the samples. Data for the baking formulas are given, the formulas being as follows:

Ingredient	Basic + 1 mg $KBrO_3$	Rich + 3 mg $KBrO_3$
Flour	100.0	100.0
Salt	1.0	1.5
Sugar	5.0	6.0
Yeast	3.0	2.0
Malt	0.0	0.25 (120°L)
Shortening	0.0	3.0
Dry Milk solids	0.0	4.0
Potassium bromate	0.001	0.003
Water (distilled)	as required	as required

In baking, standard A. A. C. C. fermentation times and temperatures were used; also National sheeting rolls for punching, and a Thompson laboratory molder for molding the loaves. Tall-form, approved pans were used. Loaves were measured for volume immediately from the

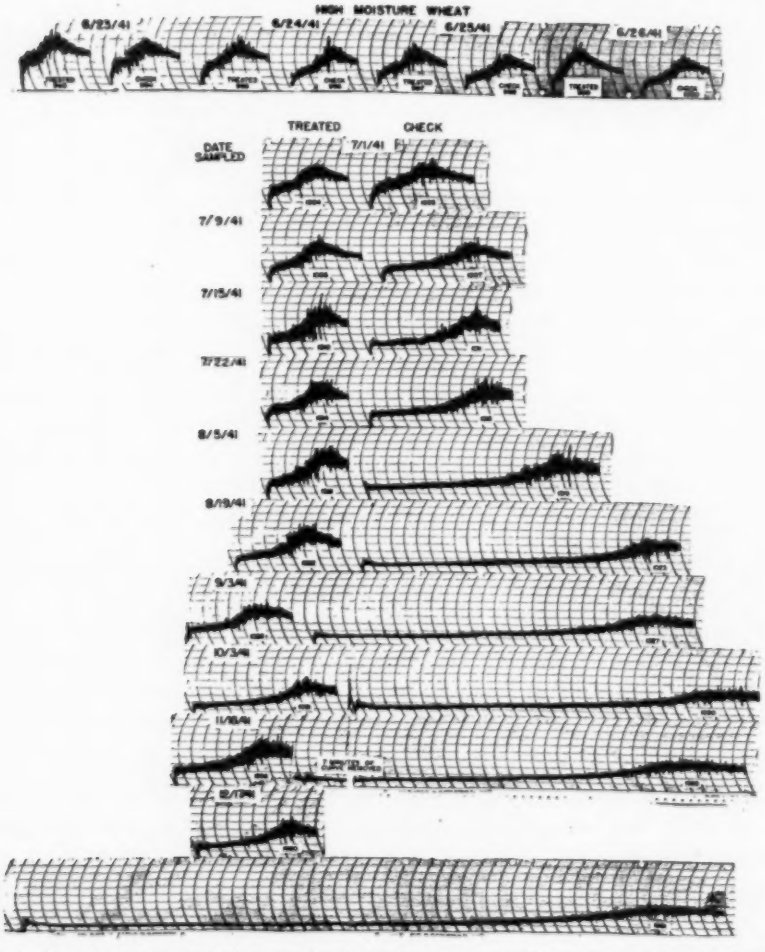

Fig. 3. Dough mixer curves (mixograms)' with flour from treated and from control (untreated) wheat.

TABLE IV

BAKING DATA FOR TREATED AND UNTREATED SAMPLES

No.	Absorption[1]	Mixing time[2]	Basic + 1 mg $KBrO_3$			Rich + 3 mg $KBrO_3$		
			Loaf volume	Grain	Crumb	Loaf volume	Grain	Crumb
		min	*cc*			*cc*		
UNTREATED—BIN 6								
993	64	2¾	658	73	—	848	95-o	95cg
995	64	2¾	645	73	—	843	95-o	95cg
997	64	2¾	553	73	—	855	98-o	98cy
999	64	2¾	618	78	—	850	98-c	95cy
1005	64	2¾	610	68	—	735	95-o	95cy
1007	64	4	687	65-o	65gy	750	93-o	95cg
1011	65	9	633	55-o	55gy	735	90-c	90gy
1015	64	10	680	60-o	70gy	735	80-c	100cy
1019	64	19	618	55-o	53gy	728	80-c	80g
1023	64	20	550	45-o	50gy	655	75-o	75gy
1027	65	30	515	45-o	65gy	650	60-o	60g
1030	62	22	400	40-o	40g	513	50-o	50g
1955	64	50	358	40-o	40g	465	40-o	40g
1961	64	50[3]	325	20-o	20gy	460	40-o	30gy
1965	64	50[3]	370	30-o	30g	440	40-o	40g
2051	64	50[3]	330	30-o	35g	475	40-o	40g
ETHYLENE TREATED—BIN 7								
994	64	2¾	673	78	—	830	98-c	98cy
996	64	2¾	653	78	—	875	98-c	95cg
998	64	2¾	663	75	—	853	98-c	95cg
1000	64	2¾	615	83	—	810	98-c	95cy
1004	64	2¾	590	83	—	810	95-o	98cy
1006	64	2¾	700	70-o	70gy	735	93-o	95cg
1010	65	5	700	70-o	70gy	730	90-c	90gy
1014	64	5	658	60-o	70gy	815	85-c	85gy
1018	64	4½	680	65-o	65gy	765	90-c	95cy
1022	63	5	638	63-o	60gy	775	90-c	88cy
1026	64	6½	710	60-o	60gy	795	85-o	85cy
1031	60	6½	675	55-o	55g	703	70-o	75g
1956	64	11	—	—	—	675	75-o	70gy
1960	64	8	625	70-o	65gy	778	78-c	80c
1964	62	7¾	608	60-c	55g	740	75-c	70cg
2052	64	9	565	50-o	50g	675	70-o	70cy

[1] Moisture basis 15%. Rich formula required 2% more than indicated.
[2] As determined by recording dough mixer (mixograph).
[3] Actually only mixed 4½ minutes in baking.

oven and scored for internal characteristics the following day. Mixing times were determined from the recording dough mixer curves, a part of the series being illustrated in Fig. 3.

It is evident that the ethylene-treated samples, with either baking formula, produced better bread than those receiving no treatment.

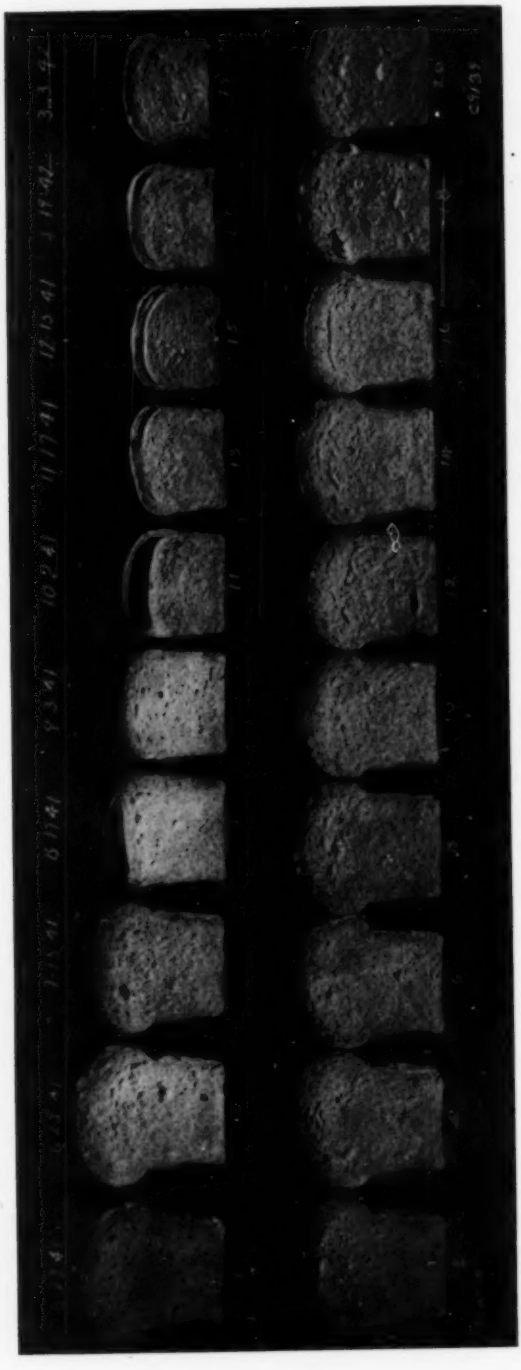

Fig. 4. Baking results with flour from treated and from untreated (control) wheat.

Experiments with normal-moisture wheat to be reported in a later communication show that treatment with ethylene also benefits the baking qualities of flour milled therefrom. As the samples aged the baking values decreased, although the decrease was much less evident after treatment with ethylene. The recording dough mixer curves (Fig. 3) show clearly the effect of damage due to storing the grain with abnormally high moisture contents. Figure 4 shows baking results of the treated and untreated wheat. All of these samples were baked in May, 1942, on the same day in order to obtain a photographic comparison. The date for the withdrawal of the samples is indicated in the figure.

Summary

Two bins each of approximately 325 bushels capacity were filled with relatively uniform high moisture wheat. During filling, one bin was treated with ethylene gas mixed in the proportion of approximately 10,000 parts of air to one of ethylene.

Application of ethylene to the freshly harvested high moisture wheat increased the rate of carbon dioxide evolution therefrom.

The treated wheat did not heat as rapidly nor as much as the untreated.

Grade of the grain, percentage germination, and baking performance of the treated samples were superior to the untreated during several months of storage.

The experiment indicates that ethylene gas does not prevent spoilage of wheat stored with a high moisture content, but that heating of such wheat may be materially retarded.

Acknowledgment

The authors wish to express appreciation to J. E. Anderson and John A. Johnson, of the Department of Milling Industry, for their assistance during the course of these investigations.

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THE ACTION OF GLUTATHIONE AND WHEAT GERM ON DOUGH IN RELATION TO PROTEOLYTIC ENZYMES IN WHEAT FLOUR

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(Read at the Annual Meeting, May 1942)

Several years ago Jørgensen, and at about the same time Balls and Hale, independently, suggested a proteolytic enzyme theory as an explanation of the improving action of oxidizing agents on dough.

Jørgensen has presented extensive experimental evidence in favor of this theory, which has since been supported by many other investigators. The present writer (1941) pointed out that when a theory has so much experimental support and approval as has been the case here, it seems justifiable to discard it only after it is definitely established that the experiments on which the theory is founded have been incorrect, or if other serious objections against it can be maintained. I further stated that new investigations with new techniques, such as those by Baker, Parker, and Mize (1942), may reveal new effects of oxidizing agents on dough constituents other than the proteolytic enzymes, that therefore the action of oxidizing agents on dough may be more complicated than was thought originally, but that such additional observations or other theories, however interesting, do not deny the now well established inhibitory action of oxidizing agents on proteolytic enzymes in flour, nor do they invalidate the Jørgensen theory.

Some objections against Jørgensen's theory have been raised (Geddes, 1941), based mainly on the behavior of glutathione and wheat germ in dough, which was interpreted as contrary to the proteolytic-enzyme theory and considered by some as of sufficient importance to eliminate it altogether. The present paper, however, reports some new evidence, which shows that the behavior of glutathione and wheat germ in dough is in perfect accord with Jørgensen's theory.

It is realized that no conclusive and final proof of the correctness of Jørgensen's theory or of any other theory on the effect of oxidizing agents in dough is available at present, and therefore one has to evaluate such a theory on the basis of accumulated experimental evidence, which thus far seems to be decidedly in favor of Jørgensen's theory. This does not exclude the possibility of actions of oxidizing agents on dough constituents other than proteolytic enzymes and it is hoped that the present paper may stimulate more work on this interesting problem.

Support of, and Objections to, Jørgensen's Theory

The very extensive experimental support of his theory by Jørgensen (1935, 1935a, 1935b, 1936, 1938, 1938a, 1939, 1939a) and the correctness of his experiments have never been questioned. Although no attempt will be made here to give a complete list of publications in support of Jørgensen's theory or confirming his experiments, the following references may be cited: Balls and Hale (1935, 1936, 1936a, 1938), Sullivan, Howe, and Schmalz (1936), Flohil (1936), L. Elion (1937), Müller (1937), Melville and Shattock (1938), E. Elion (1939), Hale (1939), Munz and Brabender (1940), Hullett and Stern (1941), E. Elion (1941), Davidson and LeClerc (1942). Furthermore E. Elion (1941) showed that Hildebrand and Burkert (1941) and Shen and Geddes (1941) also obtained results which support some of the experimental evidence put forward by Jørgensen.

Flohil (private communication) has pointed to the fact that all baking improving agents of the type in question, chemical as well as physical, depress proteolytic action, and that anything that does not depress proteolytic action is not a flour improver in the ordinarily accepted sense of the term, and he stated that *these facts cannot very well be coincidental*, although at the time this statement was made, the behavior of wheat germ and glutathione in dough still required an explanation.

The main objections against Jørgensen's theory can be briefly summarized as follows: (1) In short patent flours made from sound wheat there is such an extremely small amount of demonstrable proteolytic enzyme that it is very difficult to believe that the very profound effects obtained are associated with this minute quantity of enzyme (Baker, 1941). (2) Wheat germ and glutathione have an immediate softening effect upon dough which progressively decreases with an extension of the fermentation time, whereas papain has, as well as an immediate action, a very marked delayed softening effect. If the harmful effect of glutathione should be ascribed to activation of flour proteinases, the softening effect due to glutathione should progressively increase instead of decrease with an extension of the fermentation time (Geddes, 1941; Ford and Maiden, 1938). (3) Carbonnelle (1938), whose experiments strongly support Jørgensen's theory, nevertheless asks whether the action of glutathione is not too rapid to be attributed to enzyme activation. Sullivan, Howe, Schmalz, and Astleford (1940) state also that the action of such compounds as glutathione on dough is too rapid to be attributed solely to enzyme activation. This statement appears also in other papers, as if it were an established fact. The rapid initial softening action on dough of

relatively large additions of glutathione is an established fact indeed, but thus far we have been unable to find in the literature any experimental support for the statement that such initial action actually is too rapid to be attributed solely to enzyme action.

Purpose of the Investigation

The purpose of the present investigation has been: (1) to establish experimentally whether the action of glutathione on dough is indeed too rapid to be attributed solely to enzyme action, (2) to explain the progressive decrease of the harmful effect of glutathione on dough with an extension of the fermentation time, and (3) to evaluate critically the existing objections against the Jørgensen theory.

Action of Glutathione on Dough

Jørgensen (1936) reported the results of the addition to dough of 0.1% of glutathione and showed how this substance, which itself possesses no proteolytic activity at all, caused the dough to lose its strength very rapidly. He reported the behavior of dough made from flour and water only, and the results of farinograph and baking tests with or without the addition of 0.1% of glutathione. He concluded that flour contains powerful but latent proteolytic enzymes, which manifest their presence after the addition of an activator such as glutathione.

The flour used in Jørgensen's experiments was milled from No. 1 Manitoba wheat, and all other flours examined have shown the same behavior with added glutathione. Jørgensen, in this paper, presents strong arguments in support of the presence of powerful but latent proteolytic enzymes in sound wheat flour.

In order to establish whether or not the immediate softening action of glutathione on dough is too rapid to be attributed solely to enzyme action, we have repeated Jørgensen's tests with flour-water doughs and have compared the action of added reduced glutathione with that of added papain. As a matter of fact, results from different workers cannot be compared on an absolute basis because of the varying strength of different papain preparations, differences in purity of commercially available reduced glutathione, and variations in the behavior of different flours.

In the following tests dough has been made from distilled water and an untreated straight-grade flour milled from a 100% spring wheat. The amounts of added glutathione and papain selected were as high as possible in order to obtain a rapid initial action, but not so rapid that the doughs could not be handled just after mixing.

Simultaneously with the control test (C) two other experiments were made containing, in addition to the flour and water, 0.1% of reduced glutathione (test G) and 0.3% of papain (test P), respectively, as based on the flour weight. The glutathione and papain were added in the water that served to make the doughs.

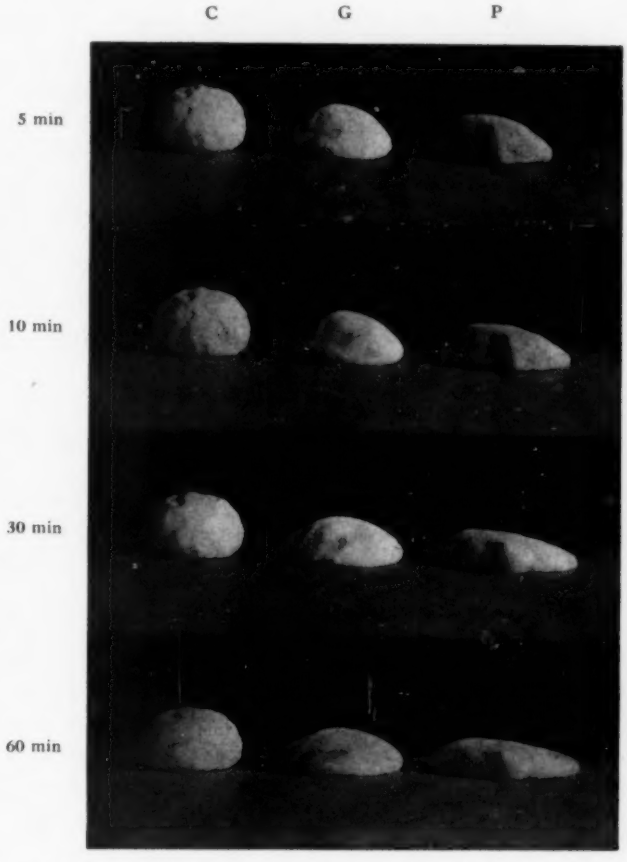

Fig. 1. Nonfermenting doughs. C = control test. G = 0.1% glutathione. P = 0.3% papain.

During mixing of both the glutathione and papain doughs a very rapid and similar softening was observed, contrary to the control test. Immediately after mixing the three doughs were rounded up and the three dough balls, which had the same size at that moment, were placed side by side on a plate and observed. It was obvious that both G and P doughs very rapidly lost their strength (Fig. 1).

The pictures in Figure 1 were taken, respectively, 5, 10, 30, and 60 minutes after the dough balls were made and they demonstrate that

the action of the papain was more *immediate*, and was stronger during the entire experiment than that of the glutathione.

In view of Baker's objections, mentioned above, the same experiments were made with a sound, untreated Southwestern *short patent* bakery-type flour. In keeping with Jørgensen's statement that all flours examined by him have shown the same behavior, this short-patent flour made from sound wheat also has shown the behavior represented in Figure 1.

These experiments confirm those of Jørgensen (1936) as far as the behavior of 0.1% of glutathione is concerned and moreover prove that the action of even 0.1% of glutathione, although very rapid, is not too rapid to be attributed solely to enzyme action, since the action of the enzyme papain has been more rapid than that of the glutathione, even in the beginning. The immediate action of added proteolytic enzyme can be even more rapid than that of glutathione, and whether the immediate action of glutathione will be slower or more rapid than that of papain seems to depend exclusively upon the relative amounts of these materials that are added to the dough.

These experiments further provide strong evidence of the presence of powerful but latent proteolytic enzymes in wheat flour, including sound short patent flour.

It might be argued, although without experimental foundation, that the effect of glutathione on dough is indeed similar to the effect of papain, provided the actions of correct amounts of both materials are compared, but that this does not prove that glutathione acts as an activator of the proteolytic enzymes present in flour. Thus the similar effects of the two substances on dough might be pure coincidence, and the glutathione might act on some other dough constituent and thus happen to produce an effect similar to that of papain. It has indeed been suggested that glutathione, papain, and other SH-containing compounds act directly on the gluten because of their common SH groups, causing the increase in fluidity of doughs.

As a matter of fact, absolute proof of any theory in this field is difficult to establish, but some additional experiments still further substantiate Jørgensen's thesis. Jørgensen (1935) has shown that yeast increases the activity of those flour proteinases which can be inhibited by bromate, and he found that if the flour had first been heated 12 hours at 95°C, which inactivates the flour proteinases, yeast no longer had such an influence. He also found that the flour proteinases which can be inhibited by bromate are activated by glutathione, but that glutathione has no such action on suspensions of heated flour. We have completed these experiments with doughs made from heated flour, prepared according to Jørgensen's method

from the flour which has been used in our experiments (Fig. 1), using exactly the same experimental procedure. The results are represented in Table I.

TABLE I

SOFTENING OF UNYEASTED DOUGHS

Addition	Flour	
	Unheated	Heated
None	No	No
0.1% glutathione	Yes	No
0.3% papain	Yes	Yes

In these experiments dough made from unheated flour was softened by both glutathione and papain addition, but dough made from heated flour was softened only by added papain and not by added glutathione. This proves that with the preliminary heating of the flour, which inactivates the flour proteinases, the material on which the glutathione acts has also been eliminated, and this increases still further the probability that glutathione acts on dough by activating the flour proteinases. If papain, glutathione and other SH-containing compounds would act directly on the gluten, they should act similarly on both heated and unheated flour.

It may of course again be argued that even our experiments with heated flour constitute nothing but coincidence, and that the glutathione still might act on some flour constituent other than proteolytic enzymes, which then also would have to be destroyed by heat; but for such a statement no experimental support would be available.

Decrease of Harmful Effect of Glutathione on Dough with Extension of Fermentation

Sullivan, Near, and Foley (1936), Sullivan, Howe, and Schmalz (1936, 1937), and Sullivan and Howe (1937) have shown that the presence of glutathione is responsible for the harmful action of wheat germ on dough, although proteolytic enzymes present in wheat germ will also have a detrimental effect. They demonstrate that when the proteolytic enzymes of wheat germ are destroyed by boiling the water extract of germ, the heated extract still has an injurious effect on dough quality, which they attribute to the fact that the heated extract of germ still contains glutathione, which, in turn, activates the latent proteinases of flour. They also state that the water extract of germ on standing loses its effect, the rate of loss being dependent on the temperature and the time. They point to the probability that the reduced glutathione (hereinafter represented by GSH), which gives a

positive nitroprusside reaction and activates proteinases, is oxidized on standing to the S-S form (hereinafter represented by GSSG), which gives no nitroprusside reaction and has no activating effect on proteolytic enzymes.

This conception has since been confirmed. Ziegler (1940, 1940a) definitely establishes the fact that only GSH has a harmful effect, and demonstrates that oxidized glutathione (GSSG) itself has an improving effect on dough, which is the most marked for an addition of about 5 mg of GSSG per 100 g of flour, *i.e.*, about 0.005% on the flour weight.

In view of these findings it must be assumed that the progressive decrease in the harmful effect of glutathione and wheat germ on dough with an extension of the fermentation time must be connected with a progressive oxidation of GSH in the dough, or a progressive disappearance of GSH from the dough through some other kind of reaction, and consequently a progressive elimination from the dough of the flour-proteinase activator GSH, which means a progressive return of the flour proteinases to their latent or inactive state and a progressive decrease of proteolytic activity in the dough to which GSH has been added, with a progressive improvement of its quality. This conception, which would explain the behavior of glutathione and wheat germ in dough in accordance with Jørgensen's theory, finds confirmation in our experiments and the available literature on this subject.

The glutathione dough G represented in Figure 1, which did not show any sign of progressive decrease from the harmful effect of added GSH, with extension of time, was analyzed 75 minutes after it was mixed and gave a strong positive nitroprusside reaction for GSH. Evidently an addition of GSH as high as 0.1% of the flour weight was not entirely oxidized or decomposed in such a relatively short time. Moreover, earlier investigators have shown that GSH oxidizes only slowly, and Ziegler (1940a) demonstrated that GSH, previously oxidized to GSSG, seems to recover its deleterious effect in a yeastless dough.

It was therefore appropriate to investigate the behavior of much smaller amounts of GSH and of papain added to flour-water doughs in the same way as in the previous experiments. To this effect tests were made, the results of which are represented in Figure 2.

C represents the control dough, made of the same flour as was used in the previous tests (Fig. 1); G was composed of the same ingredients as C with the addition of 0.005% of GSH on the flour weight, and P contains 0.03% of papain. The photographs were made 15 minutes, 30 minutes, 1 hour, and 2 hours after the dough balls were made. It appears that the whole course of the softening was con-

siderably slower, as might be expected, and that the G dough remained distinctly lower than the C dough. The P dough, however, was again lower than the G dough throughout the duration of the tests. The G dough was analyzed after two hours and no longer gave any nitroprusside reaction (although this reaction was positive immediately after mixing), showing that the small amount of added GSH

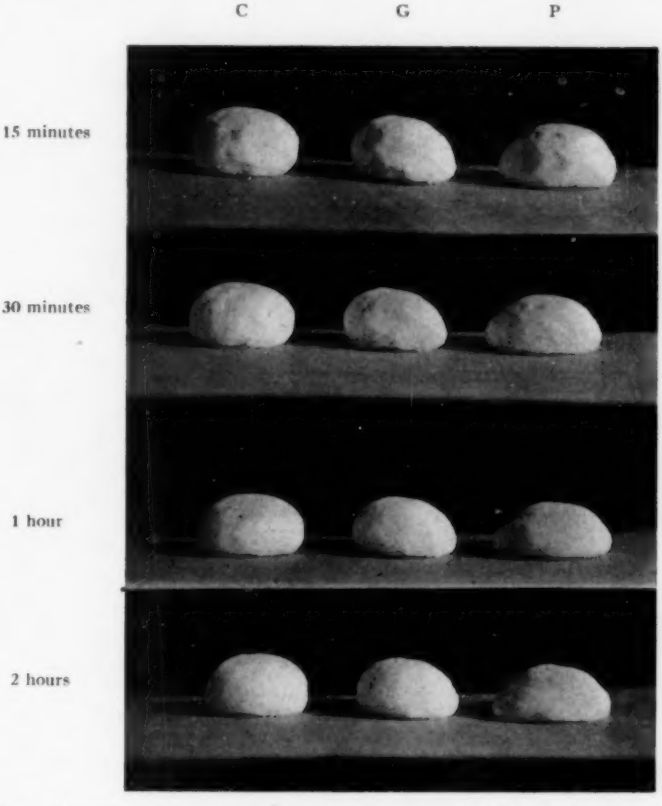

Fig. 2. Nonfermenting doughs. C = control test. G = 0.005% glutathione. P = 0.03% papain.

had completely disappeared from the dough, either by oxidation to GSSG or by some other reaction. The dough components did not prevent the nitroprusside reaction after two hours, because a further addition of a trace of GSH to the reaction mixture produced immediately a positive nitroprusside reaction.

Similar results, but more pronounced, were obtained when a control dough was compared with a G dough containing 0.005% of GSH and with a P dough containing 0.06% of papain and also when the control dough was compared with a G dough containing 0.01% of GSH and

with a P dough containing 0.12% of papain. The 0.12% papain dough had lost much more of its strength after 1 hour than the 0.06% papain dough after 2 hours, whereas the G doughs were only a little lower than the control doughs.

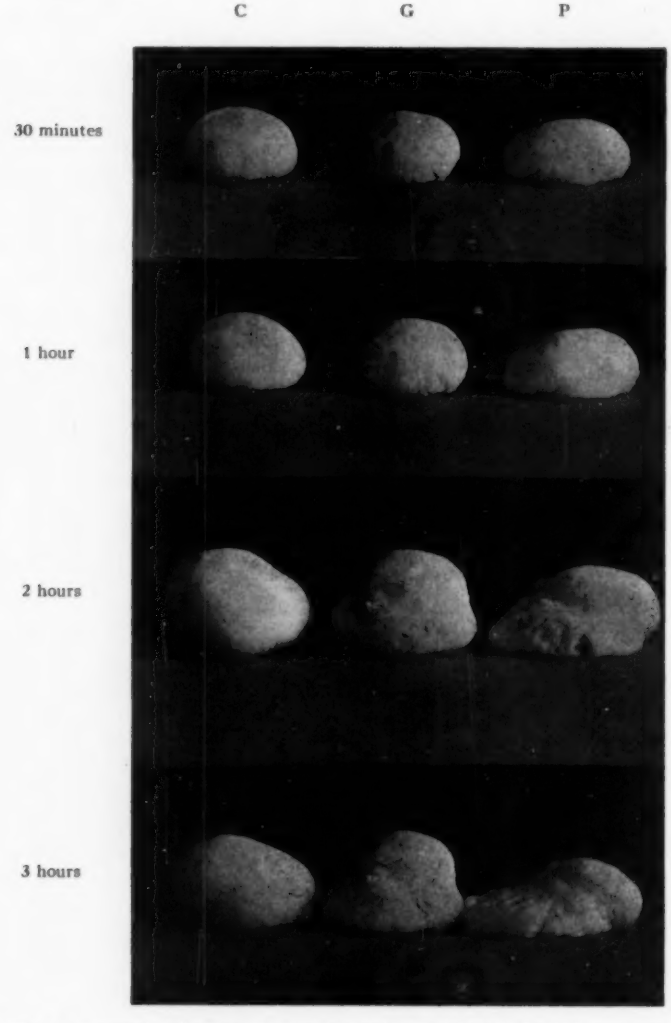

Fig. 3. Fermenting doughs. C = control test. G = 0.01% glutathione. P = 0.03% papain.

Further experiments were made with fermenting doughs, composed of the same flour and also water, yeast, dextrose, and salt. In the G dough 0.01% of GSH was added and the P dough received an addition of 0.03% of papain. The results are presented in Figure 3.

The photographs were made after 30 minutes, 1 hour, 2 hours, and 3 hours of fermentation at 30°C, respectively. In these experiments only the papain dough progressively lost much of its strength. While the glutathione dough after the beginning was slightly lower than the control dough, after 2 and 3 hours the GSH dough seemed to be equal to or better than the control.

A dough which duplicated the G dough of this experiment was analyzed 15 minutes after mixing and by that time the GSH nitroprusside reaction was found to be negative. The GSH, activator of the flour proteinases, had already disappeared and since GSSG acts as an improver according to Ziegler, it is readily understandable that the harmful effect of GSH disappeared with the extension of the fermentation time. Evidently the flour-proteinase activator disappeared rapidly from the dough, with consequently decreasing flour proteinase activity, and this may have been accompanied by the formation of GSSG, an improver, with progressive improvement of the dough with extension of fermentation time.

In a fermenting dough numerous factors collaborate to produce the final dough volume. Some of these factors, such as gas production, tend to increase the dough volume, while others, such as proteolytic activity, may tend to decrease the dough volume. If one of the dough-volume-decreasing factors is eliminated from the dough sometime during the fermentation period, without any change in the dough-volume-increasing factors, a larger dough volume must result. This fully explains the behavior of the fermenting G dough in Figure 3 as compared with the control dough C. Of course the fermenting papain dough became progressively worse with extension of time, because the added papain remained present during the entire fermentation period.

Hullett and Stern (1941) have shown that GSH may be eliminated from wheat germ by "prefermentation" and that the GSH in such a process suffers a more far-reaching change than oxidation to the GSSG form. In a normal fermenting dough the same elimination of GSH through fermentation may occur, apart from any oxidation or other decomposition of GSH which may take place. This may explain why in our experiments with fermenting dough the GSH in the G dough had disappeared so quickly and why the G dough later equaled the control. It also explains why in yeastless doughs the G test remained lower than the control.

Elimination of Remaining Objections Against Jørgensen's Theory

The experiments by Geddes (1930, 1941), Flohil (private communication), and Ford and Maiden (1938) on the behavior of glutathione in dough can now be explained in accordance with Jørgensen's theory

and in full support of it. In their tests with glutathione, or wheat germ, which contains glutathione, the softening effect in the dough occurred only in the beginning of the fermentation period and as long as sufficient GSH remained present to activate the flour proteinases. The GSH, and therewith its harmful activation of the flour proteinases, disappeared progressively from the fermenting dough with extension of the fermentation time. Some oxidation to GSSG may have had an improving influence, and with prolonged fermentation time the initial harmful effect of the added GSH might even have been overcome entirely (Flohil, private communication).

According to Ford and Maiden additions to dough of 0.005% of glutathione and 0.03% of papain had similar effects upon their doughs during the first ten minutes of mixing. It is now evident that the small amount of GSH, and therewith the flour-proteinase activator, must have disappeared very rapidly from their doughs, but not the added papain, so that these additions could not be expected to show parallel effects during the entire fermentation period, as believed by Ford and Maiden. According to Jørgensen's theory it must be expected, on the contrary, that the effect of 0.03% of papain, which remains in the dough to exert its detrimental influence, will after several hours of fermentation very far exceed the softening effect of only 0.005% of GSH, and this is precisely what Ford and Maiden found, in support of Jørgensen's theory, although they did not interpret it thus.

Baker's objections are based on the opinion that short patent flours made from sound wheat should contain only an extremely small amount of demonstrable proteolytic enzyme, making it difficult to believe that the very profound effects obtained are due to an action by this very small quantity. It follows from our experiments, in confirmation of Jørgensen (1936), that even sound short patent flours contain powerful but latent proteolytic enzymes, which only need an activator (which is supplied by glutathione or by yeast) to exert their powerful action, and it is therefore perfectly conceivable that these enzymes, once being activated, have such powerful effects.

The presence of proteolytic enzymes in flour, however, has repeatedly been reported in the literature (Stockham, 1920; Bailey, 1925; Balls and Hale, 1935; Blagoveschensky and Yurgenson, 1935; Flohil, 1936) (untreated patent flour). Landis and Frey (1938) give figures for the proteolytic activity of wheat flour as compared with malts. Their data indicate relatively appreciable proteolytic activity in different kinds of flour, including short patent flours. Similar figures have recently been published by Hildebrand (1942). Freilich and

Frey (1939) bring further evidence of proteolytic activity in patent flours.

Hale (1939), discussing the presence of proteinase in flour, states that *Jørgensen's work has definitely established the point at issue* and he shows the type of *direct evidence* that is based on the *actual extraction of the proteinase from patent flour* and the observation of its behavior in solution toward well-known activators and inhibitors of papain, which is found to be like the behavior of papain. Hale furthermore states that the unbleached patent flour used "reduced the viscosity of gelatin at about the same rate as one fifty-thousandth of its weight of crystalline papain. This quantity of enzyme seems in fact *surprisingly large*, when one considers the effect of a trace of papain added to dough. One part of commercial papain to 20,000 parts of flour may completely liquefy a dough, and a quarter of this quantity of the crystalline enzyme should also suffice. If, as seems reasonable, this marked change is caused by scarcely doubling the (flour) proteinase, it follows that *the amount naturally present is of no slight importance.* There is without doubt *enough to produce disastrous effects* if by mischance the enzyme should be activated—a situation that can conceivably arise in several ways."

Baker, Parker, and Mize (1942) refer to this paper by Hale (1939) to support their view that patent flours contain so little proteolytic enzyme that much doubt should be thrown on Jørgensen's theory, but in view of the foregoing quotations from Hale, the reference by Baker and collaborators to Hale's paper in this respect seems to be unfounded. In view of the well established fact of the presence of powerful but latent proteolytic enzymes in flour, Baker's objections are not justified.

Sullivan, Howe, Schmalz, and Astleford (1940) have made extensive studies in order to find some other action of oxidizing agents in dough, but most of their experiments gave negative results. They found that flours with poor baking quality do not always have a higher glutathione content; that the influence of bromate and similar oxidizing agents on the lipid complex of flour is not primarily responsible for their improving action; that there is no significant effect of these oxidizing agents on the diastatic enzymes, nor on the starch, nor on the sugars. If this effect is not on the proteolytic enzymes, these authors suggest that the beneficial effect of the oxidizing agents then should be upon the gluten, and upon the sulfur linkages of the gluten. However, they recognize that proteolytic enzymes themselves also contain a similar sulfur linkage and they realize that it will be difficult to prove that the activation of the proteolytic enzymes is a secondary effect. Their paper does not seem to bring direct evidence against the pro-

teolytic enzyme theory, and we do not know of other published objections to this theory which cannot be shown to be actually in accordance with it.

Velocity of the Action of Glutathione and Papain on Dough

In Table II the amounts of glutathione, wheat germ, or papain added to the dough by various authors, are summarized.

TABLE II

ADDITIONS TO DOUGH BY VARIOUS AUTHORS

Author	Papain	GSSG	GSH	Wheat germ
Geddes	—	—	—	5%
Sullivan, Howe, and Schmalz	—	—	0.02% [1]	10% [1]
Flohil	0.001%	—	—	2–4%
Ford and Maiden	0.03%	—	0.005%	—
	0.0067%	—	0.005%	—
Jørgensen	—	—	0.1%	—
Elion (Fig. 1)	0.3%	—	0.1%	—
Elion (Fig. 2)	0.03%	—	0.005%	—
	0.06%	—	0.005%	—
	0.12%	—	0.01%	—
Elion (Fig. 3)	0.03%	—	0.01%	—
Ziegler	—	0.005% (optimum)	—	—

[1] Sullivan, Howe, and Schmalz found that the initial effect on dough of 0.02% added glutathione was about the same as that of the added extract of 10% wheat germ.

We have shown that, if the proper amounts of glutathione and papain are compared, the immediate as well as the delayed actions of papain can be stronger than those of glutathione. It is also possible to select the relative amounts differently, so that both materials act with the same initial speed, whereas the papain will have a stronger ultimate effect after a prolonged period of time. The amounts may also be selected, as has been done by Ford and Maiden (0.0067% of papain and 0.005% of GSH) so that the glutathione test in the beginning gives faster action than the papain test, and later of course becomes slower.

Freilich and Frey (1939) state that "cysteine and glutathione produce immediate specific effects, which are noticeable while the dough is still being mixed, but the effects of papain are very gradual by comparison; doughs with added papain in amounts which produce *effects in bread similar* to those of cysteine and glutathione, may be normal after mixing and become soft and sticky only after a few hours' fermentation." This observation also fits Jørgensen's theory. However, the statement that glutathione produces immediate "specific" effects, whereas the effects of papain are very gradual, may easily

cause misunderstanding. This statement must have resulted from the comparison of inadequate amounts of papain and glutathione. In fact, if one compares the action on dough of papain and of GSH in amounts which produce similar effects in *bread* (that is, after the entire fermentation period, when only the original amount of papain added still retains its harmful effect, while the other ingredient (GSH) probably has already disappeared entirely from the dough) it is obvious that the initial amount of GSH added to the dough must have been considerably too large as compared with the amount of papain added, in order to have enough GSH remain in the dough to show any effect on the baked bread. Only under such abnormal conditions of comparison is the initial action of the GSH much stronger than of the papain, but this does not justify the conclusion that GSH should have any specific effect which is not shown by papain. As a matter of fact, there is no such specific difference between the action of glutathione and of papain, provided they are compared only as long as both materials are present in the dough in the amounts in which they have been added—that is, only in the very beginning of the fermentation period. When compared under such conditions, the effects of glutathione and of papain on dough are entirely similar, as shown in our experiments.

Chemical Effects of Flour Proteinases

Proteolytic enzymes can be measured only by the effects which they produce. As far as the chemical changes involved in such action are concerned, Bailey (1925, page 266) stated: "It is conceivable that substantial modification of the gluten proteins in contact with active proteases may result without any material increase in the simpler degradation products of proteolysis which can be estimated by analytical methods now available."

Blagoveschensky and Yurgenson (1935) showed that flour proteinases possess a definite solvent action on wheat proteins, which effect is one of disaggregation and not of increase in amino nitrogen.

Balls and Hale (1936, 1936a) also show that very considerable modification of a protein can occur and still be recorded only as a small change when measured by any of our present-day chemical methods.

Notwithstanding the analytical difficulties to be expected, Amos (1942) recently found a small but definite and progressive increase even in amino nitrogen in an unyeasted dough.

The fact that the strong physical influence of proteolytic enzymes in flour on the dough properties is difficult to establish with chemical methods may have been the cause for the belief that the amount of proteolytic enzymes in flour must be insignificant.

Summary

The action of glutathione and of wheat germ, which contains glutathione, on dough has been considered by some authors as being evidence against the correctness of Jørgensen's theory of the action of bromates and similar oxidizing agents as flour improvers. The present author, from the literature and new experimental evidence, shows that the hitherto unexplained behavior of glutathione in dough can be explained, in accordance with and in support of Jørgensen's theory, solely by the activating effect of reduced glutathione upon the powerful but latent flour proteinases and that the progressive decrease of the harmful effect of reduced glutathione with increased fermentation time results either from the progressive oxidation of reduced glutathione to oxidized glutathione (which is not a proteinase activator, but a flour improver) or from its progressive elimination from the dough because of some more far-reaching change.

It is pointed out that, while oxidizing agents may be found to act also on dough constituents other than proteolytic enzymes, the proteolytic enzyme theory of Jørgensen is too well founded and supported to permit its rejection.

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OBSERVATIONS ON THE pH OF CHEMICALLY LEAVENED PRODUCTS

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Interest in the pH of chemically leavened products has been revived by the increasing knowledge of the effect of H-ion concentration on thiamin retention. Variables affecting the determination of pH of baked products are of new significance because these may influence the correlation of thiamin destruction with pH.

Several investigators have studied the measurement of pH of flour and baked products. Halton and Fisher (1928) found that pH of flour extracts increased with increase in the ratio of water to flour used in the determination. Whittier and Grewe (1929) substantiated the effect of the water-to-sample ratio and concluded that pH of extracts of baked products is not a reliable measure of H-ion concentration of the undiluted product. Garnatz (1937) found that pH of water extracts decreased on standing and Stamberg and Bailey (1939) found that pH of cakes shifted toward neutrality on standing three days.

Biscuits and cakes were made by the formulas and procedures shown in Tables I and II. Typical leavening mixtures were used in

TABLE I

BISCUIT FORMULAS

	Acid leavening				
	Sodium acid pyrophosphate	Monocalcium phosphate, anhydrous	Monocalcium phosphate, hydrated	Cream of tartar	MCP, 36.3% SAS, 63.7%
Neutralizing value of acid	70.0	83.5	80.0	50.0	86.8
	g	*g*	*g*	*g*	*g*
Flour: 50% patent soft wheat, chlorine bleached, pH 5.0–5.2	200.0	200.0	200.0	200.0	200.0
Salt (NaCl)	4.0	4.0	4.0	4.0	4.0
Soda ($NaHCO_3$)	2.5	2.5	3.0	3.0	3.0
Acid leavening	3.57	3.0	3.75	6.0	3.45
	ml	*ml*	*ml*	*ml*	*ml*
Shortening (Wesson Oil)	25.0	25.0	25.0	25.0	25.0
Milk (10% reconstituted DSM)	122.0	122.0	122.0	122.0	122.0

Procedure: Dough mixed in Kitchen-aid ½ minute; rolled, folded, rolled, and cut. Baked 12 minutes at 460°F in electric oven.

TABLE II

CAKE FORMULAS

Flour: 50% patent soft wheat, chlorine bleached	260	g
Sugar: fine granulated	250	g
Hydrogenated shortening	65	g
Dried skim milk	30	g
Water	200	ml
Whole fresh egg	82	g
Salt	4	g
Soda	3	g
Acid leavening:		
Sodium acid pyrophosphate	4.3	g
or monocalcium phosphate—anhydrous	3.6	g
or monocalcium phosphate—hydrated	3.75	g
or cream of tartar	6.0	g
or MCP 36.3% + SAPP 63.7%	3.45	

Procedure: (1) The milk was reconstituted in the water before using. (2) Sugar and shortening creamed 5 minutes on second speed. (3) Egg added and creamed 5 minutes on second speed. (4) Dry ingredients sifted 4 times and added alternately with milk at first speed in 30 seconds. (5) Mixed second speed 1½ minutes. (6) Scaled at 325 g and baked 35 minutes at 400°F in an electric oven.

amounts usually employed for each particular combination. Except where noted, the pH of biscuits and cakes was determined by the following procedures.

Method of Determining pH

Biscuit: A whole biscuit that had been out of the oven 3 to 4 hours was weighed, broken up in a porcelain mortar, and twice the weight of the biscuit in ml of water was added. The whole was macerated with the pestle until a uniform mixture was obtained and the biscuit was thoroughly wetted. A portion of this slurry was placed in a 20-ml beaker and pH determined directly on the slurry with a Leeds and Northrup 1199–12 glass electrode.

Two readings were made with stirring of the slurry between readings. If readings deviated beyond 0.1 pH a third reading was made. Poor checks indicated improper sample preparation.

To avoid contamination of the electrode, it was washed with distilled water after each determination and occasionally wiped with lens paper or cleansing tissue. Sometimes acetone was used to remove adhering fat.

Cake: Cakes were allowed to stand overnight after baking. The crust was cut off and 15 g of crumb was crumbled in a mortar. 30 ml water was added and this mixed thoroughly with a pestle. From here, procedure was the same as for biscuits.

Biscuits

pH of biscuit doughs: An attempt was made to determine the pH of the straight doughs containing typical leavening mixtures. Because of large error in the determinations on the undiluted dough it was found necessary to resort to diluting the dough with half its weight of water. In Table III typical values are given for biscuit doughs containing the more usual leavening mixtures.

TABLE III

pH OF BISCUIT DOUGHS

(Electrometric on mixture of 1 part water to 2 parts dough)

	Average of twelve	Range	PE of mean
MCP-SAS "combination"	6.95	6.57–7.20	±0.0361
Monocalcium phosphate, hydrated	6.79	6.58–6.95	±0.0256
Sodium acid pyrophosphate	6.74	6.30–7.05	±0.0345
Monocalcium phosphate, anhydrous	6.66	6.48–6.78	±0.0180
Cream of tartar	6.39	6.18–6.80	±0.0323
Unleavened dough	5.82	5.78–5.91	±0.0105

All the leavening combinations raised the pH of the dough, cream of tartar the least and an MCP-SAS combination the most. Differences between hydrated monocalcium phosphate and sodium acid pyrophosphate and between sodium acid pyrophosphate and anhy-

drous monocalcium phosphate were not statistically significant; all other differences were significant.

Age and temperature of dough vs pH of biscuit: When biscuit doughs were allowed to stand there was no significant change in pH. This may have been influenced by the relatively large error in the determination on the dough. However, the pH of biscuits with some of the leavening mixtures was affected by the time the dough stood before baking. These values are shown in Table IV.

TABLE IV

EFFECT OF AGE AND TEMPERATURE OF DOUGH ON pH OF BAKED BISCUIT
(Electrometric pH on mixture of 2 parts water to 1 part whole biscuit)

Acid leavening	Age of dough			
	0 hr	2 hrs	4 hrs	Over-night
DOUGHS HELD AT 20°–23°C				
Sodium acid pyrophosphate	7.49	7.22	7.10	6.98
Monocalcium phosphate, anhydrous	6.86	6.85	6.86	6.86
Monocalcium phosphate, monohydrate	6.85	6.88	6.86	6.83
Cream of tartar	6.65	6.85	6.74	6.66
Monocalcium phosphate, hydrated, 36.3% / Sodium aluminum sulfate, 63.7%	7.06	7.04	6.97	6.91
No leavening	5.98	—	—	—
DOUGHS HELD AT 8°C				
Sodium acid pyrophosphate		7.39	7.36	7.12
Monocalcium phosphate, anhydrous		6.85	6.84	6.88
Monocalcium phosphate, monohydrate		6.88	6.86	6.86
Cream of tartar		6.81	6.72	6.73
Monocalcium phosphate, hydrated, 36.3% / Sodium aluminum sulfate, 63.7%		7.02	7.02	6.93

Biscuits made with sodium acid pyrophosphate were more acid the longer the dough was allowed to stand, and the higher the dough temperature the greater the change. An MCP-SAS combination showed the same tendency to a lesser extent. Cream of tartar biscuits appeared to go through a maximum in about 2 hours and then declined; this trend was shown by three individual doughs from which these data were developed.

From Tables III and IV, it is apparent that the pH of biscuits was higher than that of the doughs from which they were made and that this difference varied with the type of leavening used.

Age and condition of biscuit vs pH of biscuit: It is common practice to air-dry and crumble samples which are to be assayed for vitamin content and the assay thus obtained is sometimes related to pH of

the same sample and sometimes to the pH of the fresh undried product. In order to determine whether pH of the dried and undried material is the same, pH determinations were made on fresh and on one-day-old whole biscuits and on air-dried, crumbled, whole biscuits.

TABLE V

EFFECT OF AGE AND CONDITION OF BISCUIT ON pH OF BISCUIT
(Electrometric pH on mixture of 2 parts water to 1 part whole biscuit)

	Aged 1 hr, whole	Aged overnight		Overall change
		Whole	Crumbled	
Sodium acid pyrophosphate	7.50	7.40	7.22	−0.28
Monocalcium phosphate, anhydrous	6.80	6.81	6.74	−0.06
Monocalcium phosphate, hydrated	6.84	6.85	6.72	−0.12
Cream of tartar	6.70	6.72	6.60	−0.10
MCP-SAS combination	7.01	7.05	6.90	−0.11

The results in Table V show that on aging the exposed whole biscuit there is no significant change in pH. When the biscuit is crumbled and air-dried there is a definite drop in pH which varies with the leavening. Furthermore, the difference in pH between the aged, whole, and crumbled biscuits increases with increase in pH of the whole fresh biscuit. This is shown in Figure 1.

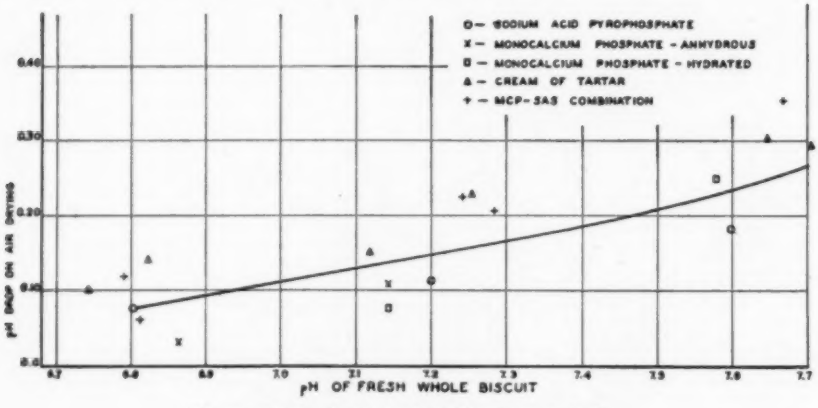

Fig. 1. Change in pH of whole biscuit on air drying.

Effect on pH of water-to-sample ratio: It has been demonstrated that increasing the proportion of water to sample used in the determination results in an increase in pH which is not accounted for by dilution effect (Whittier and Grewe, 1929). In order to determine whether this is true with all common leavening combinations, pH was determined on fresh whole biscuits made with the various leavenings

and by varying the water-to-sample ratio as shown in Table VI. These data show that pH increases on all typical biscuits with increase in the proportion of water used in the test. Only in the case of the

TABLE VI

EFFECT ON pH CAUSED BY VARIATION IN RATIO OF WATER TO BISCUIT USED IN THE DETERMINATION

	Ratio water to whole biscuit			
	1 : 1	2 : 1	4 : 1	10 : 1
Sodium acid pyrophosphate	7.40	7.49	7.58	7.64
Sodium acid pyrophosphate, change in 1 hr	−0.13	−0.15	−0.18	−0.04
MCP-SAS combination	6.88	7.01	7.05	7.20
Monocalcium phosphate, anhydrous	6.78	6.82	6.90	6.96
Monocalcium phosphate, hydrated	6.70	6.83	6.90	6.97
Cream of tartar	6.61	6.68	6.65	6.72

SLURRY VS FILTERED EXTRACT—(10 : 1)

	Slurry	Filtered extract		
		15 min	30 min	60 min
Sodium acid pyrophosphate	7.64	7.64	7.62	7.56
Sodium acid pyrophosphate, change in 1 hr	—	—	—	−.08
Monocalcium phosphate, anhydrous	7.08	7.06	7.08	7.04
Monocalcium phosphate, hydrated	7.04	7.08	7.06	7.06

biscuits made with sodium acid pyrophosphate did the slurries decrease in pH on standing one hour.

The official A. O. A. C. and A. A. C. C. methods, which call for a 10-water-to-1-sample ratio, give generally high pH values on chemically leavened products. The filtered extract and the unseparated slurry give essentially the same result.

TABLE VII

pH OF BISCUIT CRUMB AND CRUST

(Reconstituted dry skimmilk as liquid)

	Whole biscuit	Crumb	Top crust	Bottom crust
Sodium acid pyrophosphate	7.50	7.72	7.11	7.11
MCP-SAS combination	6.90	7.20	6.58	6.70
Monocalcium phosphate, hydrated	6.88	6.91	6.72	6.70
Monocalcium phosphate, anhydrous	6.84	6.93	6.62	6.70
Cream of tartar	6.60	6.98	6.20	6.26

EFFECT OF BROWNING ON PH OF CRUST

	Pale crust	Brown crust
Sodium acid pyrophosphate	7.03	6.72
Monocalcium phosphate, anhydrous	6.97	6.64

pH of biscuit crumb and crust: The pH of bread and cake is usually determined on the crumb but since biscuits have a relatively high proportion of crust it has been the practice in this laboratory to determine pH on the whole biscuit by the method previously described.

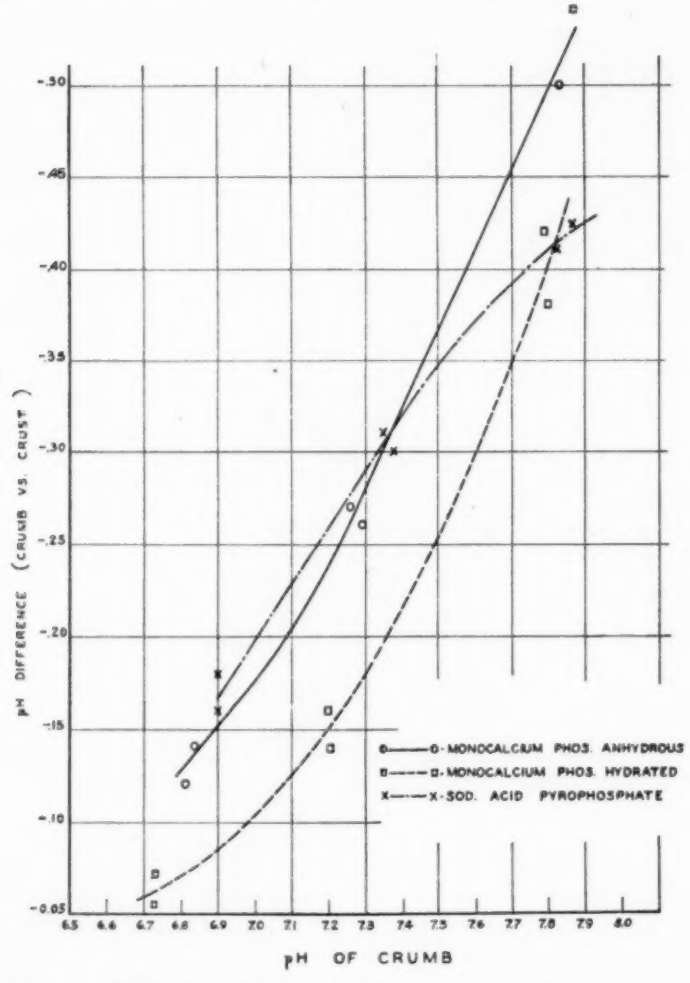

Fig. 2. Differences between the pH of biscuit crust and crumb at various levels.

In order to establish the differences in the pH of different portions of biscuits leavened with various mixtures, determinations were made on the biscuit crumb, the top and bottom crusts, and the whole biscuit. The results in Table VII show that the crumb was more alkaline and the crust more acid than the whole biscuit. The bottom and top crusts were the same.

The difference in pH of the crust and crumb varied with the leavening, the browning of the crust, and the pH of the crumb. At usual pH levels, calcium acid phosphates showed the least difference and cream of tartar the most. The browner the crust the more acid. Using water instead of milk raised the pH 0.2 to 0.3 unit with every leavening except cream of tartar; with the latter the pH was 0.05 to 0.10 lower.

The effect of increase in alkalinity of the crumb upon the difference in pH between the crust and crumb is shown for the phosphates in Figure 2. The more alkaline the crumb, the greater the difference between the crust and crumb. Anhydrous monocalcium phosphate gave a relatively more acid crust than the monohydrate but the change with increase in pH of the crumb was essentially the same for both of these salts. Sodium acid pyrophosphate at low crumb-pH had the most acid crust but as alkalinity of the crumb increased the difference in pH of crust and crumb changed less than with the calcium phosphates.

Cakes

pH of batters and cakes: It is shown in Table VIII that as with biscuit doughs, the usual leavening salts raised the pH of the batter, and cakes generally showed a higher pH than the corresponding batters.

TABLE VIII

pH OF BATTERS AND CAKES

(Electrometric, on batter as is)

	Age of batter			
	0 hr	2 hrs	4 hrs	Overnight
BATTERS HELD AT 23°C				
Sodium acid pyrophosphate	7.19	6.91	6.85	6.98
Monocalcium phosphate, anhydrous	7.00	6.20	6.30	6.50
MCP-SAS combination	6.72	6.64	6.72	6.91
Monocalcium phosphate, hydrated	6.32	6.36	6.36	6.78
Cream of tartar	6.30	6.20	6.22	6.33
No leavening	6.13	—	—	—
CAKES FROM ABOVE BATTERS				
Sodium acid pyrophosphate	7.28	—	—	7.13
Monocalcium phosphate, anhydrous	6.84	—	—	6.78
MCP-SAS combination	6.98	—	—	6.88
Monocalcium phosphate, hydrated	6.84	—	—	6.68
Cream of tartar	6.88	—	—	6.76

On letting the batters stand, all but the one made with monohydrate monocalcium phosphate showed an initial drop in pH which with sodium acid pyrophosphate was relatively retarded; on prolonged standing, pH of the batter tended to rise. The cakes from the aged batters were more acid than those from fresh batters.

Effect on pH of water-to-sample ratio: Increasing the proportion of water to cake crumb used in the pH determination gave an effect similar to that found with biscuits. Since the cake crumb was more moist than a biscuit, pH measurements were also made on the crumb without added water. Although the error in the latter values is large the results are shown with the other data in Table IX to demonstrate the pronounced effect of the first increment of water added.

TABLE IX

EFFECT ON pH CAUSED BY VARIATIONS IN RATIO OF WATER TO CAKE CRUMB

	Ratio water to crumb				
	0	1:1	2:1	4:1	10:1
Sodium acid pyrophosphate	6.30	7.20	7.27	7.38	7.38
MCP-SAS combination	6.55	6.87	6.98	7.06	—
Monocalcium phosphate, anhydrous	6.02	6.82	6.90	6.99	7.03
Cream of tartar	6.47	6.71	6.88	6.94	—
Monocalcium phosphate, hydrated	6.10	6.66	6.84	6.88	—
No leavening	6.38	—	6.60	—	—

pH of various parts of cake: The pH determinations on various portions of cakes containing typical leavening mixtures are shown in Table X. The cake was most alkaline in the middle and became progressively more acid toward the crust. Unlike biscuits, the bottom crust was more acid than the top crust.

TABLE X

pH OF CAKE CRUMB AND CRUST

(Electrometric on 2 parts water to 1 part cake portion)

	Middle of cake	Whole crumb	Half inch from crust	Top crust	Bottom crust
Sodium acid pyrophosphate	7.26	7.25	7.24	6.70	6.12
MCP-SAS combination	7.01	6.98	6.90	6.50	5.88
Monocalcium phosphate, anhydrous	6.88	6.88	6.79	6.67	6.01
Cream of tartar	6.98	6.84	6.77	6.29	5.72
Monocalcium phosphate, hydrated	6.76	6.76	6.69	6.58	6.11

Summary and Conclusions

The pH values for biscuit doughs, cake batters, and baked products containing the more common leavening mixtures were determined

and the effect of certain variations in the method of determining pH were studied.

Biscuit doughs did not change in pH on standing. Biscuit baked from doughs leavened with sodium acid pyrophosphate and with a typical combination of monocalcium phosphate and sodium aluminum sulfate became more acid the longer the dough stood before baking. The air-dried crumbled biscuit was more acid than similar whole biscuit of the same age. The higher the water-to-sample ratio used in the pH determination the higher was the pH. The biscuit crumb was the most alkaline and the crust the most acid; the relation of pH of crust to crumb changed with the pH of the crumb.

The more liquid cake batters changed in pH on aging, and the older the batter the lower was the pH of the cake baked from it. The effect of changing ratio of water to sample and the relative levels of pH in different portions of the cake were similar to those found on biscuits.

Aside from variations in baking procedure which have been shown to influence the pH of the baked product as measured by a given method, variations in the method of determining pH on the same biscuit or cake had a pronounced effect on the value obtained. High values were obtained on a fresh center crumb with 10 parts water to 1 part sample in the determination; low values resulted when the whole air-dried product was tested with the minimum of water. These extremes in procedure can result in a difference of 0.4 to 0.6 pH units on the same baked product, the magnitude of the difference depending upon the leavening.

An estimate of the retention of thiamin in baked products cannot be made on the basis of pH unless the method employed in determining pH is standardized and has been previously correlated with vitamin assays.

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REDUCTION OF THE FERMENTABLE CARBOHYDRATE CONTENT OF CORN BY KILN DRYING

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Many distilleries include in their grain specifications the stipulation that corn shall have a moisture of not more than 12% to 13%. Much of the new corn crop each year is kiln-dried, both to meet the distillery specifications and to avoid spoilage in the elevator. Plant observations over a period of several years have revealed a drop in alcohol yield in the autumn, which appears to coincide with the use of kiln-dried corn. In order to determine the effect of kiln drying on the fermentable carbohydrate content of corn, a number of samples were obtained from an experimental commercial drier.

These portions of the same original batch had been dried at 160°, 170°, 180°, 190°, and 200°F. Our procedure was to set small-scale laboratory fermenters of spirits mash (92% corn, 8% barley malt) under conditions similar to those used in mashing and setting plant fermenters. The corn was ground, cooked atmospherically for one hour, and then pressure-cooked for one hour in the autoclave at 22 pounds. The cooks were cooled to 145°F and a malt slurry added for conversion. The converted mash was cooled to 68°–72°F, 20% stillage was added to adjust the concentration to 38 gallons per bushel of grain,

TABLE I

Alcohol Yield from Corn Dried in an Experimental Commercial Drier

Sample No.	Drying temperature	Gallons 100 proof alcohol per bushel of grain (dry basis)
	°F	
6	160	6.07
7	170	6.09
8	180	6.08
9	190	6.14
10	200	5.82

and the pH adjusted to 4.8. Fermenters were then set with an inoculum of 2% yeast by volume. At the end of 68 hours' fermentation the alcohol was distilled off and read on the Zeiss refractometer. Alcohol yield was calculated as gallons of 100 proof alcohol per bushel of grain on the dry basis and as plant fermentation efficiency, which is equal to the grams of alcohol actually obtained on distillation divided

by the theoretical alcohol yield, which is based on the initial total sugar present in the set fermenter.

It will be noted that the yield from the corn dried at 200°F was significantly lower than the yield from the portions dried at lower temperatures (Table I). In order to investigate this problem more fully an experimental rotary drier was constructed in our laboratory (Fig. 1). This drier was installed in an incubator equipped with

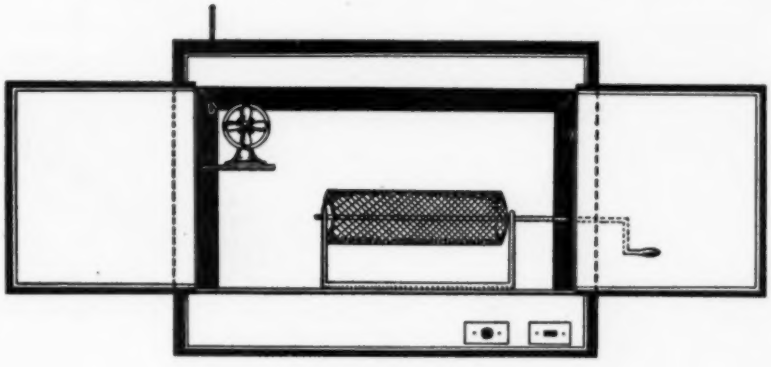

Fig. 1. Rotary drier.

heaters capable of maintaining a temperature as high as 240°F. An electric fan in the incubator insured even heat distribution. The drier was rotated at approximately 30 rpm. Samples of new-crop corn with high moisture were secured and portions were dried in the experimental drier at 180°, 190°, 200°, 210°, 220°, and 230°F. The length of time for drying was varied to secure the desired final moisture.

The corn used in the experiment reported in Table II had an initial moisture of only 14%, so it was dried to a moisture of 10%. It will be noted that the proof gallons per bushel yield recovered from corn dried at 210° and 230°F was somewhat lower than the yield obtained

TABLE II

ALCOHOL YIELD FROM CORN WITH AN INITIAL MOISTURE OF 14% DRIED IN ROTARY LABORATORY DRIER (FIG. 1)

(Initial moisture 13.47%)

Sample No.	Drying temp.	Drying time	Final moisture	Gallons 100 proof alcohol per bushel of grain (dry basis)
	°F	min	%	
1	170	50	9.89	5.83
2	180	40	10.14	5.86
3	190	20	10.71	5.94
4	210	20	10.45	5.68
5	230	20	9.57	5.74

from corn dried at lower temperatures. It was felt that a sample of higher moisture corn should be tested, since corn reaching a commercial elevator with 14% moisture would not normally be dried.

Accordingly a sample of corn was secured with a moisture of 16%. Again the alcohol yield from corn dried at temperatures higher than 200°F was lower than the yields from corn dried at lower temperatures (Table III).

TABLE III

ALCOHOL YIELD FROM CORN WITH AN INITIAL MOISTURE OF 16% DRIED IN ROTARY LABORATORY DRIER (FIG. 1)

Sample No.	Drying temp.	Drying time	Final moisture	Gallons 100 proof alcohol per bushel of grain (dry basis)
	°F	min	%	
1	Unheated control		14.13	6.04
2	180	15	12.19	6.00
3	190	13	12.08	5.92
4	200	12	12.44	6.01
5	208	10	12.22	5.78
6	220	8	12.36	5.79

TABLE IV

ALCOHOL YIELD FROM CORN WITH AN INITIAL MOISTURE OF 17% DRIED IN ROTARY LABORATORY DRIER (FIG. 1)

Sample No.	Drying temp.	Drying time	Final moisture	Gallons 100 proof alcohol per bushel of grain (dry basis)
	°F	min	%	
1	Unheated control		17.28	6.31
2	180	19	14.22	6.12
3	190	17	13.97	6.12
4	200	16	13.87	6.02
5	210	14	13.71	5.90
6	220	12	14.27	5.92

Table IV presents the results of test fermentations on a corn sample with an initial moisture of 17%. Larger amounts of this corn were used in each drying than were used in previous experiments. For this reason, a longer exposure to the heat was necessary to attain the desired final moisture. Several important indications may be found in these data: first, the detrimental effect of drying temperatures above 200°F; second, the unheated control gave a higher proof gallon per bushel yield than any of the kiln-dried samples. These results were checked by additional experiments employing the same conditions.

In order to approximate more closely the conditions employed in certain commercial driers, a different type of laboratory kiln drier was constructed (Fig. 2).

Compressed air is passed through a manometer in order to maintain a constant rate of flow. The air is then passed through a glass coil where it is heated by a Bunsen burner to the desired temperature.

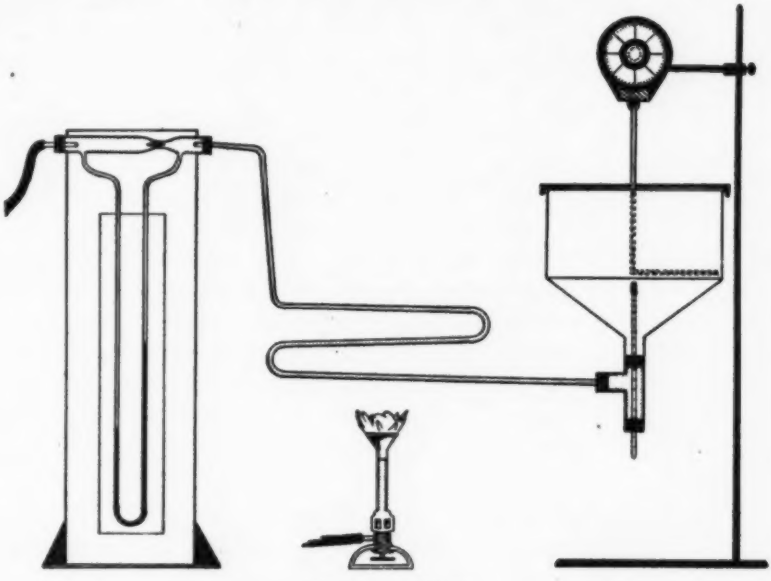

Fig. 2. Drier No. 2.

Temperature is determined by means of a thermometer inserted in the bottom of the large Buchner funnel, which serves as the drier. Corn, placed in the funnel, is stirred by a bent-glass rod driven by a small laboratory motor. A metal cover over the funnel reduces heat loss.

TABLE V

ALCOHOL YIELD FROM CORN DRIED IN LABORATORY DRIER NO. 2 (FIG. 2)

Sample No.	Drying temp.	Drying time	Final moisture	Gallons 100 proof alcohol per bushel of grain (dry basis)
	°F	min	%	
1	160	45	12.70	6.14
2	180	35	12.78	6.10
3	200	33	12.53	6.19
4	210	30	12.85	6.18
5	Unheated control		15.76	6.35

The corn used in the experiment reported in Table V had an initial moisture of 17.5%. The amount of corn and the velocity of air were varied so that 45 minutes were required to dry the sample to a moisture of 12.5% at 160°F. This time-temperature relationship is the same

TABLE VI

ALCOHOL YIELD FROM CORN DRIED IN LABORATORY DRIER NO. 2 (FIG. 2)

Sample No.	Drying temp.	Drying time	Final moisture	Gallons 100 proof alcohol per bushel of grain (dry basis)
	°F	min	%	
1	160	45	13.50	6.17
2	180	35	13.63	6.16
3	200	33	13.61	6.20
4	210	30	13.46	6.21
5	220	29	13.14	6.21
6	Unheated control		16.87	6.30

as that used by at least one commercial drier. It will be noted that the yield of alcohol from the unheated control was again higher than that from any of the kiln-dried samples.

Table VI presents the results of an experiment carried out under the same conditions as the previous one and merely serves to confirm those results. The decrease in yield between corn dried at temperatures lower than 200°F and corn dried at 200°F and higher was not evident with this type of drier. The explanation may lie in the fact that the first type was inclosed in a cabinet and there was no significant temperature differential between the corn and the surrounding atmosphere.

TABLE VII

ALCOHOL YIELD FROM CORN DRIED IN LABORATORY DRIER NO. 2 (FIG. 2) USING REDUCED DRYING TIME

Sample No.	Drying temp.	Drying time	Final moisture	Gallons 100 proof alcohol per bushel of grain (dry basis)
	°F	min	%	
1	160	23	13.13	6.14
2	160	23	13.13	6.15
3	200	17	13.10	6.16
4	200	17	13.10	6.17
5	220	15	13.10	6.18
6	220	15	13.10	6.18
7	Unheated control		16.11	6.29
8	Unheated control		16.11	6.28

TABLE VIII

ALCOHOL YIELD FROM CORN DRIED AT ROOM TEMPERATURE

Sample No.	Drying temp.	Final moisture	Gallons 100 proof alcohol per bushel of grain (dry basis)
		%	
1	Room temperature	12.16	6.07
2	Undried control	15.62	6.07

With the second type the heat loss was much greater and the grain actually never reached the temperature of the air passed through it. This might seem to be a defect in the laboratory drier but in reality it corresponds to the conditions in at least one commercial kiln drier.

In order to determine the effect of time of exposure to the drying temperature on alcohol yield, the amount of corn used in each drying was reduced so that the desired final moisture could be obtained in half the time required in previous experiments. Results of fermentations run on this corn revealed that the yields from the dried portions were still all lower than the yield from the control (Table VII).

In order to determine whether or not the reduction in yield noted above could be due merely to loss of moisture from the kernel and not to the temperature of drying, a sample of 17.5% moisture corn was divided into two portions. One was dried at room temperature to 12% moisture and the other used as the undried control. The proof-gallons-per-bushel yield of alcohol obtained upon fermentation of these samples was identical (Table VIII).

Discussion

Two effects of kiln drying have been found. The first of these is the decrease in yield of 0.1 to 0.2 proof gallon per bushel from all kiln-dried corn. This effect appears to be relatively independent of reduction of the length of exposure to the drying temperature. Evidently some chemical or physico-chemical changes occur which alter the fermentable carbohydrate portion of the grain. The most feasible explanation is that the action of heat on the starch produces a certain amount of unfermentable dextrins. That this change is due to heat and not to dehydration of the kernel has been proved by fermentation tests on corn dried at room temperature. The point might be raised that various types of grain might react differently. However, in the course of this investigation numerous samples from various sources were tested and in no instance was there any indication that flinty character, starch content, or any other factor influenced the results. In the work reported here there was no attempt to conduct a survey of types of corn in relation to the effect of kiln drying. Such a study might yield valuable information.

It is difficult or impossible to avoid purchasing kiln-dried corn. In such cases careful consideration should be given to the type of drier used and the temperature at which it is operated.

Our experimental work indicates that under certain conditions drying at high temperatures results in a still further decrease in alcohol yield. As stated before, this effect was noted with the first type of laboratory drier used but not with the second. With the first type

the corn was heated to the indicated temperature rapidly and was maintained at that temperature for the duration of the drying period. With the second type the stream of heated air was maintained at the indicated temperature but heat loss to the atmosphere was so great that the temperature of this corn was always 15 to 20 degrees lower. In plant-scale operations this differential is probably much smaller. The decrease noted in alcohol yield from corn dried in an experimental commercial drier at temperatures above 200°F indicates that at least some commercial driers produce an effect similar to the first type of laboratory drier reported in this paper.

Summary

Kiln drying of corn results in a 2% to 3% decrease in alcohol yield.

Under certain conditions, kiln drying of corn at temperatures of 200°F and above may result in a decrease of 4% to 6% in the alcohol yield.

Since the effect of kiln drying varies with the type of equipment used, it should be advantageous to the grain companies and to the manufacturers of kiln-drying equipment to institute a thorough program of research on the problem. The most feasible plan would be to conduct fermentation studies on corn dried in laboratory-scale models of the various types of commercial driers.

The action of heat on the fermentable carbohydrate portion of the grain has not been definitely characterized but is probably a formation of a certain amount of unfermentable dextrins.

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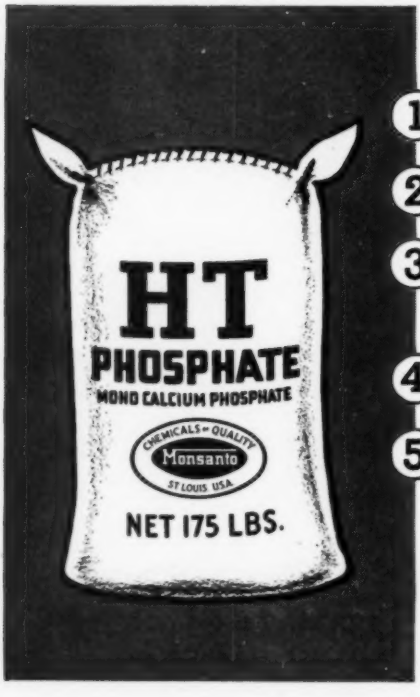

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